ADVANCES IN POLYMER SCIENCE

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II Evaluation of Multicomponent Microencapsulation Systems

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Water Soluble Polymers for Immunoisolation I: Complex Coacervation and Cytotoxicity

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Seventy five synthetic, semi-synthetic, natural and biological water soluble polymers have been evaluated as potential biomaterials for cell and islet immunoisolation. Measurements have included the cytotoxicity of polyanion and polycation solutions towards insulinoma cells as well as the type of complex coacervate interaction produced. These results have been coupled with metrics delineating the quality of the capsular membrane produced and correlated with molecular properties of the individual polymers tested. Microcapsules prepared from over one thousand binary polyelectrolyte combinations have been characterized according to their mechanical strength, capsule shape, surface smoothness, stability, and swelling or shrinking. Based on this screening 47 pairs have been identified as alternatives to the standard poly-L-lysine-alginate chemistry. The quality of the membrane produced was observed to be a strong function of the polymer molecular weight, as well as the solution concentration. Additionally, the ionic content of the backbone, the chemistry and location of functional group attachment, the chain rigidity, aromaticity, conformation and extent of branching were identified as important variables in the type of complex produced. The presence of secondary hydrogen bonding interactions was also found to be significant. Processing conditions such as the type and concentration of the simple electrolyte, the pH, the reaction time and surface coating have also been investigated.

Keywords: Bioartificial pancreas, biomaterials, complex coacervation, immunoisolation, microencapsulation, polyelectrolytes, water soluble polymers.

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1 Introduction

Water soluble polymers include naturally occurring polysaccharides [1], biomolecules such as DNA, semi-synthetic species such as modified cellulose, as well as synthetic molecules, predominantly based on radical polymerization of acrylic monomers [2]. At present their principal applications are as hydrocolloids in food additives [3], in environmental applications such as municipal water treatment [4] and for resource recovery and processing [5]. The market for water soluble polymers is now several billion dollars per annum, with growth rates in consumption of 5–8% exceeding that of most sectors in the chemical industry. Over the past thirty years, considerable research interest has been dedicated to the utilization of water soluble and swellable polymers in biological applications. These include opthalmological devices [6], matrices for controlled drug delivery [7, 8], dental materials and scaffolds for tissue regeneration [9, 10]. They can also be utilized for the formation of immunoisolation barriers [11]. The latter involves the production of semi-permeable membranes by either a phase inversion process [12] or a complex coacervation reaction [13].

The principal issues involved in developing polymeric biomaterials are biodegradability and biocompatibility. While degradation can be quantified relatively precisely [14], a definition of biocompatibility has been elusive. At present, one can only refer to the suitability of a material for a specific application in a given site within the body. Furthermore, polymers which will contact blood have much more stringent requirements since they can often provoke a stronger immune system response. Unfortunately some polymers which have shown good compatibility, such as polyethylene oxide, have very poor mechanical properties. To compensate for this, two general approaches are employed. In some instances, mechanically suitable copolymers have been used to produce devices such as an artificial heart [15, 16] and are then surface coated to attempt to prevent a host system response [17]. The major limitation in this regard is the difficulty in obtaining complete surface coverage and the reversibility of adsorption. An alternative approach is to synthesize biomaterials from polymers which have intrinsically good biocompatibility, for the purpose at hand, and to avoid the necessity of coating. It is this latter philosophy to which the authors of this paper subscribe. Therefore we have been motivated to evaluate both the material properties and compatibility of polyelectrolytes as perspective immunoisolation barriers.

Several competing strategies for immunoisolation such as vascular grafts [18], hollow fibres [19] and both macro- [20,21] and microencapsulation [22–24] have been evaluated over the past two decades. These have been discussed in several recent reviews [25, 26]. The primary advantages of microencapsulation are that it avoids the necessity of major surgery, and the use of a complex coacervation reaction facilitates the investigation of alternative polymer chemistries. The separation of cells into several thousand particles also provides additional security in that some microcapsules can fail, or be rejected, without subjecting the entire population to risk. The application of polymers as immunoisolation barriers includes the development of a bioartificial liver [27, 28] and bioartificial parathyroid [29]. Water soluble or swellable macromolecules are also used for pain control for terminal cancer patients [30], in the treatment of Alzheimer's [31] and neurological disorders [32], and in the encapsulation of pancreatic islets.

The development of biological microencapsulation systems has included pioneering efforts by Chang [33], Lim and Sun [34] and Sefton and Broughton [35]. The latter two have focused on the immunoisolation of pancreatic islets for the formation of a bioartificial pancreas. Thin film polymer membranes comprised of water-insoluble thermoplastics, symplexes and hydrogel copolymers have been prepared, and several recent reviews detail the technological aspects involved in cell or islet encapsulation [36–38]. Unfortunately the fragile nature of islets, and the specificity of the capsule processing conditions to the properties of the often viscoelastic polymer fluid, have limited the number of polymers which have been rigorously evaluated (Table 1). Indeed, most researchers have been limited to the poly-L-lysine-alginate [35] and alginate-chitosan [55] systems which are based on the ionotropic gelation of alginate with polyvalent cations, typically calcium. However, although lysine-alginate produces quite stable membranes, it has relatively poor mechanical properties. Ionotropic gelling alterna-

Table 1. Summary of nonionic and ionogenic water soluble polymers utilized for encapsulation

Membranes Prepared Via Coacervation		Gelling Agent/	Ref.
Inner Polymer (Core)	External Polymer (Receiving Bath)	Template	
Alginate	Polyvinylamine	Calcium	39
Alginate	Polyvinylamine	Calcium	40
Alginate	Protamine	–	41
Alginate	Spermine	–	42
Alginate	Polybrene	Barium	43
Cellulose Sulfate	Polydiallyldimethyl ammonium chloride	–	44
Carboxymethylcellulose	Chitosan	–	45
Carboxymethylcellulose	Diethylaminoethyldextran	–	45
Carrageenan-κ	Chitosan	Potassium	46
Chitosan	Alginate	Calcium	47
Chitosan	Pentasodiumtripoly-phosphate hexahydrate	–	48
Chitosan	Xanthan	–	49
Chondroitin Sulfate A	Chitosan	–	45
Chondroitin Sulfate C	Spermine	–	43
Heparin	Protamine	–	50
Hyaluronic Acid	Chitosan	–	45
Pentasodiumtripoly-phosphate hexahydrate	Chitosan	–	51
Polyacrylates/Methacrylates (anionic)	Polyacrylates (cationic)	–	52
Polyphosphazene (anionic)	Polylysine	Calcium	53
Polystyrene Sulfonate	Polybrene	Agarose	54

tives for alginate, as an inner polymer, have thus far been limited to the cationic chitosan and blends of alginate with other polysaccharides such as carrageenan, carboxymethylcellulose or dextran sulfate [56]. Furthermore, it has been speculated that a family of capsule chemistries will need to be available in order to provide alternatives in the event that the primary immunoisolation material is rejected by a given patient. This problem is likely to be particularly acute for Type-I diabetics, since they typically contract the disease for over 40 years. Therefore, in an attempt to identify alternatives to the classical systems listed in Table 1, we have undertaken a massive screening of polyelectrolytes in an attempt to make molecular inferences as to the complexation mechanism. The evaluation has included 35 polyanions and 40 polycations in 1235 binary combinations (Table 2).

Table 2. Polyelectrolytes utilized in this screening

#	Polymer type and molecular weight grade (if applicable)	Brand name	Concentration tested (wt %)	Supplier
	Naturally occurring polyanions			
1a	Alginate (Sodium), High	Keltone HVCR	0.2–2.0	Kelco/Merck, San Diego, CA
1b	Alginate (Sodium), Low	Keltone LV	0.2–2.0	Kelco/Merck, San Diego, CA
1c	Alginate (Sodium), Low	Manugel DMB	0.2–2.0	Kelco/Merck, San Diego, CA
1d	Alginate (Sodium), Low–Medium–High	–	0.2–2.0	Sigma, St. Louis, MO
1e	Alginate (Sodium), Low	UP LVG	0.2–2.0	Pronova Biopolymer, Drammen, Norway
1f	Alginate (Sodium) Medium	UP MVG	0.2–2.0	Pronova Biopolymer, Drammen, Norway
1g	Alginate (Sodium), High–Low	Kelcoloid HVF-LVF	0.2–2.0	Kelco/Merck, San Diego, CA
2	Alginate (Proplylene Glycol Modified), Medium–High	Protanal SD-H, PVH-A	1.0–2.0	Pronova Biopolymer, Drammen, Norway
3	Carboxymethyl Amylose	–	0.5–2.0	Sigma, St. Louis, MO
4a	Carboxymethyl Cellulose (Sodium), Low–Medium–High	–	0.5–2.0	Sigma, St. Louis, MO
4b	Carboxymethyl Cellulose (Sodium), Medium	7MF	0.5–1.5	Aqualon/Hercules, Wilmington, DE
5	Carboxymethyl Dextran	–	1.0–15.0	Fluka, Ronkonkoma, NY
6a	Carrageenan-ι	Gelcarin GP-379 NF	0.2–1.0	FMC Corp., Newark, CT
6b	Carrageenan-κ	Gelcarin GP-911 NF	0.2–1.0	FMC Corp., Newark, CT
6c	Carrageenan-λ	–	0.5–1.5	Fluka, Ronkonkoma, NY
6d	Carrageenan-κ, Low	Aubygel X52	0.5–1.5	Sanofi Bio-Industries, Paris, France
7	Cellulose Sulfate (Sodium)	–	0.2–2.0	Janssen Chimica, Geel, Belgium
8	Chondroitin 4-Sulfate (Sodium)	A	0.2–1.0	Sigma, St. Louis, MO
9	Chondroitin 6-Sulfate (Sodium)	C	0.2–1.0	Sigma, St. Louis, MO
10	Dextran Sulfate, 500 kDa	–	1.0–10.0	Pharmacia, Uppsala, Sweden
11	Gellan Gum (Deacetylated)	Kelcogel	0.6 in 0.3% Hexa-monophosphate	Kelco/Merck, San Diego, CA
12	Gum Arabic	–	1.0	Sigma, St. Louis, MO
13	Heparin, 3 KDa	–	1.0–5.0	Sigma, St. Louis, MO
14a	Hyaluronic Acid, 1–2000 kDa	–	0.1–5.0	Genzyme, Cambridge, MA

Table 2. (continued)

#	Polymer type and molecular weight grade (if applicable)	Brand name	Concentration tested (wt %)	Supplier
14b	Hyaluronic Acid	FCH	0.1–1.0	Pronova Biopolymer, Drammen, Norway
15a	Pectin (Low Esterified)	–	1.0–5.0	Sigma, St. Louis, MO
15b	Pectin (Low Esterified)	315 NHND	1.0–5.0	Sanofi Bio-Industries, Paris, France
16	Polygalacturonic Acid	–	1.0–5.0	Sigma, St. Louis, MO
17a	Xanthan, High	Rhodigel	0.2–2.0	R.T. Vanderbilt, Norwalk, CT
17b	Xanthan, High	Ticaxan	0.2–2.0	TIC Gums, Belcamp, MD
17c	Xanthan, High	Ketrol T/TF	0.5–1.5	Kelco/Merck, San Diego, CA
Synthetic Polyanions				
18	Pentasodiumtripolyphosphate hexahydrate	–	1.0–10.0	Sigma, St. Louis, MO
19	Polyacrylamide (70% Carboxy Modified), 200 kDa	–	1.0–5.0	Polysciences, Warrington, PA
20	Polyacrylamide (90% Carboxy Modified), 200 kDa	–	1.0–5.0	Aldrich, Milwaukee, WI
21	Polyacrylamide-*co*-Acrylic Acid, 10 and 40% Carboxylated	–	1.0–5.0	Polysciences, Warrington, PA
22	Polyacrylamido-2-methyl-1-propanesulfonic Acid	–	1.0–5.0	Aldrich, Milwaukee, WI
23a	Polyacrylic Acid, 2.1,6.10,20,60,140,250, 450 kDa	–	1.0–5.0	Polysciences, Warrington, PA
23b	Polyacrylic Acid, 450,750,1000,4000 kDa	–	0.1–1.0	Aldrich, Milwaukee, WI
23c	Polyacrylic Acid (Modified)	–	0.1–1.0	Gelest, Tullytown, PA
24	Polyglutamic Acid, 5–30 kDa	–	1.0–5.0	Gelest, Tullytown, PA
25	Polymaleic Acid	–	1.0–5.0	Polysciences, Warrington, PA
26	Polymaleic Anhydride	–	1.0–5.0	Polysciences, Warrington, PA
27	Polymethacrylic Acid (Sodium) 15 kDa	–	1.0–2.0	Polysciences, Warrington, PA
28	Polymethylvinylethermaleicacid 20–70 kDa	–	1.0–5.0	Polysciences, Warrington, PA
29	Polymethylvinylethermaleicacid Anhydride, 50, 70 kDa	–	1.0–5.0	Scientific Polymer Products, Ontario, NY

Table 2. (continued)

#	Polymer type and molecular weight grade (if applicable)	Brand name	Concentration tested (wt %)	Supplier
30	Polystyrene Sulfonic Acid (Sodium), 70 kDa	–	1.0–5.0	Polysciences, Warrington, PA
31	Polyvinylphosphate	–	1.0–10.0	Polysciences, Warrington, PA
32	Polyvinylphosphonic Acid	–	1.0–2.0	Polysciences, Warrington, PA
33	Polyvinylsulfone (Anionic)	–	1.0–10.0	Polysciences, Warrington, PA
34	Polyvinylsulfonic Acid (Sodium) 2 kDa	–	1.0–10.0	Polysciences, Warrington, PA
Naturally Occurring or Biological Polycations				
35a	Chitosan Glutamate, Medium	Protasan HV	0.5–2.5	Pronova Biopolymer, Drammen, Norway
35b	Chitosan Glutamate, Low	Protasan LV	0.5–2.0	Pronova Biopolymer, Drammen, Norway
36	Chitosan (Glycol Modified), 80 kDa	–	0.5–2.0	Wako Chemicals, Richmond, VA
37	Dextran (Diethylaminoethyl Modified), 500 kDa	–	1.0–10.0	Pharmacia, Uppsula, Sweden
38	Hydroxyethyl Cellulose Trimethylamine (Quaternary)	JR-125	0.05–0.5	Amerchol, Edison, NY
39	Lysozyme	–	1.0–5.0	Sigma, St. Louis, MO
40	Poly- l-Lysine (Hydrobromide) 30–70 kDa	–	0.1–1.0	Sigma, St. Louis, MO
41	Salmine Sulfate, 5–10 kDa	–	1.0–5.0	Fluka, Ronkonkoma, NY
42a	Protamine Sulfate, 5–20 kDa	Grade III	1.0–5.0	Sigma, St. Louis, MO
42b	Protamine Sulfate	–	1.0–5.0	Fluka, Ronkonkoma, NY
Synthetic Polycations				
43a	Polyacrylamide (Cationic)	492C, 496C	0.05–0.3	Cytec, Wayne, NJ
43b	Polyacrylamide (Cationic)	Jayfloc 3468	0.1–0.5	Callaway, Columbus, GA
44	Polyacrylamide-*co*-Methacryloxyethyltrimethyl-ammonium Bromide, 80/20	–	1.0–5.0	Polysciences, Warrington, PA
45a	Polyallylamine Hydrochloride, 60 kDa	–	1.0–5.0	Polysciences, Warrington, PA
45b	Polyallylamine Hydrochloride, 10, 57 kDa	–	1.0–5.0	Aldrich, Milwaukee, WI
46	Polyamide (Cationic), 100 kDa	Discostrength 5807, Discol 792-A	0.1–0.5	Callaway, Columbus, GA

Table 2. (continued)

#	Polymer type and molecular weight grade (if applicable)	Brand name	Concentration tested (wt %)	Supplier
47	Polyamine	4030	1.0–5.0	Callaway, Columbus, GA
48	Polyamine (Quarternary), dimethylamine/ epichlorohydrin	Agefloc B50	1.0–5.0	CPS Chemicals, West Memphis, AK
49	Polybrene (hexamethrine bromide)	–	1.0–5.0	Sigma, St. Louis, MO
50	Polybutylacrylate-*co*-Methacryloxyethyl Trimethylammonium Bromide (80/20)	–	1.0–5.0	Polysciences, Warrington, PA
51	Poly-3-chloro-2-hydroxypropylmethacryl-oxyethyl dimethylammonium Chloride	–	1.0–5.0	Polysciences, Warrington, PA
52a	Polydiallyldimethylammonium Chloride, Low & High	Agefloc WT and PC Series, Agequat 400	0.5–5.0	CPS Chemical Co., West Memphis, AK
52b	Polydiallyldimethylammonium Chloride, 240 kDa	17338	0.5–5.0	Polysciences, Warrington, PA
53	Polydiallyldimethylammonium Chloride-*co*-Acrylamide, 75/25, 50/50	Agequat C3204, C505, 5008	1.0–5.0	CPS Chemical Co., West Memphis, AK
54	Polydiallyldimethylammonium Chloride-*co*-N-Isopropyl Acrylamide	–	1.0–5.0	Synthesized by R. Pelton, McMaster Univ.
55	Polydimethylamine-*co*-epichlorohydrin (Quaternary), 25,75 kDa	652	1.0–5.0	Aldrich, Milwaukee, WI
56	Polydimethylamine-*co*-epichlorohydrin (Quaternary)	–	1.0–5.0	Scientific Polymer Products, Ontario, NY
57a	Polydimethylaminoethylacrylate-*co*-Acrylamide (Quaternary)	–	0.1–0.5	Synthesized in our laboratory
57b	Polydimethylaminoethylacrylate-*co*-Acrylamide (Quat.), 88/12	–	0.05–0.5	Betz Laboratories, Trevose, PA
58	Polydimethylaminoethylmethcrylate-*co*-Acrylamide (Quat.), 81/19, 9,100 kDa	–	0.05–0.5	Betz Laboratories, Trevose, PA
59	Polydimethylaminoethylmethacrylate (Quaternized)	–	1.0–5.0	Polysciences, Warrington, PA
60	Polydimethylaminoethyl Methacrylate (Acryloxy, Quaternized)	–	1.0–5.0	Polysciences, Warrington, PA

Table 2. (continued)

#	Polymer type and molecular weight grade (if applicable)	Brand name	Concentration tested (wt %)	Supplier
61	Polyethyleneimine, 2,25,40,70,80 kDa	G35 SG, Waterfree SG, Luviquat FC 905/550	0.1–10.0	BASF, Parsippany, NY
62	Polyethyleneimine-Epichlorohydrin Modified, 20 kDa	634	1.0–5.0	Scientific Polymer Products, Ontario, NY
63	Polyethyleneimine (hydroxyethylated), 50,70 kDa	–	1.0–5.0	Polysciences, Warrington, PA
64	Polyethyleneimine (80% ethoxylated), 50,70 kDa	–	1.0–5.0	Scientific Polymer Products, Ontario, NY
65	Poly-2-hydroxy-3-methacryloxypropyl Trimethylammonium Chloride	–	1.0–5.0	Polysciences, Warrington, PA
66	Poly-2-hydroxy-3-methacryloxyethyl Trimethylammonium Chloride	–	1.0–5.0	Polysciences, Warrington, PA
67	Polyhdroxyproplymethacryloxy Ethyldimethyl Ammonium Chloride	–	1.0–5.0	Polysciences, Warrington, PA
68	Polyimadazoline (Quaternary), Oligomer	653	1.0–5.0	Scientific Polymer Products, Ontario, NY
69	Poly-2-methacryloxyethyltrimethylammonium Bromide, 50,200 kDa	–	1.0–5.0	Polysciences, Warrington, PA
70	Polymethacryloxyethyltrimethylammonium Bromide/Chloride	–	1.0–5.0	Polysciences, Warrington, PA
71	Polymethyldiethylaminoethylmethacrylate-*co*-acrylamide 81/19	3200 kDa	0.05–0.5	Betz Laboratories, Trevose, PA
72	Poly-1-methyl-2-vinylpyridinium Bromide, 50 kDa	–	1.0–5.0	Polysciences, Warrington, PA
73	Poly-1-methyl-4-vinylpyridinium Bromide, 50 KDa	–	1.0–5.0	Polysciences, Warrington, PA
74	Polymethylene-*co*-Guanidine Hydrochloride, Oligomer	654	0.2–2.0	Scientific Polymer Products, Ontario, NY
75	Polyvinylamine, 20,70,220 kDa	–	0.1–2.0	Air Products, Allentown, PA
76	Poly-N-vinylpyrrolidone-*co*-Dimethylaminoethyl-methacrylate (Quaternary), High	–	1.0–5.0	Polysciences, Warrington, PA
77	Poly-4-vinylbenzyltrimethylammonium Chloride, 100,400 kDa	707	1.0–5.0	Scientific Polymer Products, Ontario, NY
78	Poly-4-vinylbenzyltrimethylammonium Chloride	–	1.0–5.0	Polysciences, Warrington, PA

1.1
Polymer-Polymer Interactions

Solutions containing two polymers undergo several types of interactions which can ultimately lead to phase separation. These include (a) simple coacervation (incompatibility) which produces two phases of approximately equal volume, and (b) complex coacervation where the polymers are concentrated in a gel or precipitate phase with the supernatant essentially polymer free. The complex coacervation of two charged or nonionic polymers has been shown to be important in membrane formation [57]. In addition to electrostatic effects, secondary interactions such as hydrogen bonding (with a force of 4–6 kcal/mol), van der Waals forces (approximately 1 kcal/mol), as well as charge transfer and hydrophobic interactions can contribute to the stability of the membrane. When one of the polymers is in excess a (c) soluble complex or "sol" is typically formed. The particular nature of the polymer-polymer interaction is dependent on the concentration and density of interacting groups. Complexation is also known to be a function of the molecular weight and solution pH and ionic strength. Generally, polyelectrolytes with high charge densities interact to form precipitates. In most cases, the complex coacervation reaction is stoichiometric beyond a certain chain length (usually a few hundred) [58]. Therefore, the ratio of the interacting species is important. The rate of complexation can be of the order of fractions of a second [59], although the kinetics are reduced with increasing molecular weight. The morphology of the reaction product (precipitate, gel) is also sensitive to the kinetics and time of formation.

2
Experimental

2.1
Identification of Polymers for the Screening

In selecting potential polymers for screening four requirements were established: (1) the polymer must be soluble in water and physiological solutions since organic solvents are, in many cases, cytotoxic; (2) the polymers should have either permanent or pH inducible charges; (3) the primary side chain functional groups should not be known to induce immune system responses; (4) the polymers must either gel in the presence of ions of the opposite charge (chelation) or participate in coacervation reactions. In general, polymers which required additives, such as crosslinking agents, to enhance the membrane formation were not considered. Polymers were selected which contained anionic and cationic charges derived from various functionalities. Additionally, the molecular weight range was varied from oligomeric to several million daltons. Where possible, and in particular for synthetic polymers, the charge spacing within a given polymer was varied to test the effect of charge spacing on the membrane formation. The screening was designed to test an equal number of synthetic and naturally occurring polyanions and polycations. Therefore, approximately twenty candidate

polymers were selected from each of these four categories with the exception of naturally occurring polycations for which relatively few species are readily available.

2.2 Polymer Solution Preparation and Purification

All polymers utilized in this investigation have been listed in Table 2, along with their supplier and the concentration range over which they were tested. Polymers were either used as received or purified by filtration through a 0.22 or 0.45-μm Millipore cellulose acetate membrane. For aseptic applications autoclaving was carried out for 20 min at a temperature of 121 °C. Qualitative properties of each polymer are listed in Table 3. For polymers supplied as solutions, dialysis was carried out in membranes (Spectrum Medical Industries, Houston, TX) with a MWCO of 10,000 daltons.

Polymer solutions were prepared by dispersing the polymer powder in a saline solution prepared with distilled deionized water. Following complete dispersion in the vortex of the fluid the samples were agitated under mild conditions (< 100 RPM) until the solution was homogeneous. For some solutions the dissolution was so rapid that the agitation step could be eliminated. The polymer viscosities were then measured using a Ubbelohde viscometer. The pH of the polymer solutions was adjusted using dilute acetic acid and sodium hydroxide. Some polymers were supplied as liquids and were subsequently diluted with distilled deionized water to the appropriate concentration.

2.3 Polymer Solution Specifications

In order to generate data which could subsequently be utilized for islet encapsulation, specific screening conditions were required. Therefore, all polymer solutions were prepared in a pH range between 5 and 8, a temperature between 20 and 25 °C and an ionic strength which mimicked the physiological solutions required for cell survival. Specifically, the pH was generally kept between 5 and 6 for polycations to permit the dissociation of, for example, tertiary amines. The polyanions, which are generally the preferred candidates for cell suspension fluids, were tested at pHs between 6 and 7 for cell viability reasons. In most cases polymer solutions were prepared by dissolving a powder in phosphate buffer solution (PBS) so as to allow for a convenient osmotic pressure for the cells. Additionally, the viscosities of the two polymeric solutions (nominally one polyanion and one polycation) were kept within a range (<150 cPs) which would be required for the processing of droplets. This generally limited the maximum polymer concentration which could be tested to 1–2 wt % for the polyanions and 1–5% for the polycations, with specific concentrations for all polymers listed in Table 2.

Table 3. Properties of polymers used in this investigation

Polymer	Molecular weight[a]	Charge	Charge Density[b]	Chain Conformation	Backbone	Functional Group Attachment	Hydrogen Bonding
Naturally Occurring Polyanions							
Alginate (Sodium)	Medium	Induced	Medium	Rigid	Cyclic	Side Chain	Strong
Alginate (Propylene Glycol Modified)	Medium	Induced	Medium	Rigid	Cyclic	Side Chain	Moderate
Carboxymethyl Amylose	Medium	Induced	Medium	Rigid	Cyclic	Side Chain	Strong
Carboxymethyl Cellulose	Medium-High	Induced	Medium	Rigid	Cyclic	Side Chain	Strong
Carboxymethyl Dextran	Medium	Induced	Medium	Rigid	Cyclic	Side Chain	Strong
Carrageenan	Medium	Induced	Medium	Rigid	Cyclic	Side Chain	Strong
Cellulose Sulfate	Medium	Permanent	Medium	Rigid	Cyclic	Side Chain	Strong
Chondroitin 4-Sulfate	Medium	Permanent	Medium	Rigid	Cyclic	Side Chain	Strong
Chondroitin 6-Sulfate	Medium	Permanent	Medium	Rigid	Branch/Cycl	Side Chain	Strong
Dextran Sulfate	Medium	Permanent	Medium	Rigid	Cyclic	Side Chain	Strong
Gellan Gum	Medium	Induced	Medium	Rigid	Cyclic	Side Chain	Strong
Gum Arabic	Medium	Induced	Medium	Rigid	Cyclic	Side Chain	Strong
Guar Gum	Medium	Induced	Medium	Rigid	Cyclic	Side Chain	Strong
Heparin	Low	Permanent	Medium	Rigid	Cyclic	Side Chain	Strong
Hyaluronic Acid	High	Permanent	Medium	Rigid	Cyclic	Side Chain	Strong
Pectin	High	Induced	Medium	Rigid	Cyclic	Side Chain	Strong
Xanthan	High	Induced	Medium	Rigid	Cyclic	Side Chain	Strong
Synthetic Polyanions							
Pentasodiumtripolyphosphate Hexahydrate	Oligo	Induced	High	Flexible	Linear	Backbone	Weak
Polyacrylamide (Carboxy-Modified)	High	Induced	Medium	Flexible	Linear	Side Chain	Moderate
Polyacrylamide-*co*-Acrylic Acid	High	Induced	Medium	Flexible	Linear	Side Chain	Moderate
Polyacrylamido-2-methyl-1-propanesulfonic Acid	High	Permanent	Medium	Flexible	Linear	Side Chain	Moderate
Polyacrylic Acid	Low-High	Induced	High	Flexible	Linear	Side Chain	Moderate

Table 3. (continued)

Polymer	Molecular weight[a]	Charge	Charge Density[b]	Chain Conformation	Backbone	Functional Group Attachment	Hydrogen Bonding
Polyacrylic Acid (Modified)	High	Induced	Medium	Flexible	Linear	Side Chain	Moderate
Polyglutamic Acid	Low	Induced	High	Flexible	Linear	Side Chain	Moderate
Polymaleic Acid	Medium	Induced	Medium	Flexible	Linear	Side Chain	Strong
Polymaleic Anhydride	Medium	None	Low	Flexible	Linear	Side Chain	Strong
Polymethacrylic Acid	Medium	Induced	Medium	Flexible	Linear	Side Chain	Moderate
Polymethylvinylether Maleic Acid	Low	Induced	Medium	Flexible	Linear	Side Chain	Moderate
Polystyrene Sulfate	Medium	Permanent	High	Flexible	Linear	Side Chain	Weak
Polyvinylphosphate	Medium	Permanent	High	Flexible	Linear	Side Chain	Weak
Polyvinylphosphonic Acid	Low	Permanent	High	Flexible	Linear	Side Chain	Weak
Polyvinylsulfate	Medium	Permanent	High	Flexible	Linear	Side Chain	Weak
Polyvinylsulfonic Acid (Sodium)	Low	Permanent	High	Flexible	Linear	Side Chain	Weak
Naturally Occurring Polycations							
Chitosan	High	Induced	Medium	Rigid	Cyclic	Side Chain	Strong
Chitosan (Glycol Modified)	High	Induced	Medium	Rigid	Cyclic	Side Chain	Strong
Chitosan (Quaternary)	Medium	Permanent	Medium	Rigid	Cyclic	Side Chain	Moderate
Dextran (Diethylaminoethyl Modified)	High	Induced	Medium	Rigid	Cyclic	Side Chain	Strong
Hydroxyethyl Cellulose Trimethylamine (Quaternary)	High	Permanent	Medium	Rigid	Cyclic	Side Chain	Strong
Lysozyme	Low	Induced	Medium	Flexible	Globular	Side Chain	Strong
Poly-L-Lysine	Low-Medium	Induced	High	Flexible	Linear	Backbone	Strong
Protamine Sulfate/Salmine Sulfate	Low	Induced	High	Flexible	Linear	Backbone	Strong

[a] Oligo (10^3), Low (10^4), Med. (10^5), High (10^6)
[b] Low (0-33 mol %), Medium (34-66 mol %), High (67-100 mol %).

Table 3. (continued)

Polymer	Molecular weight[a]	Charge	Charge Density[b]	Chain Conformation	Backbone	Functional Group Attachment	Hydrogen Bonding
Synthetic Polycations							
Polyacrylamide (Cationic) #1	High	Permanent	Medium	Flexible	Linear	Side Chain	Weak
Polyacrylamide (Cationic) #2	High	Permanent	Medium	Flexible	Linear	Side Chain	Weak
Polyacrylamide-*co*-Methacryloxy Propyltrimethyl Ammonium Br	Medium	Permanent	Medium	Flexible	Linear	Side Chain	Moderate
Polyallylamine	Low	Induced	High	Flexible	Linear	Backbone	Moderate
Polyamide (Cationic)	High	Induced	High	Flexible	Linear	Backbone	Moderate
Polyamine	High	Induced	Medium	Flexible	Linear	Backbone	Moderate
Polyamine (Quaternized)	Low	Permanent	High	Flexible	Linear	Backbone	Weak
pButylacrylate-*co*-Methacryloxyethyl Trimethylammonium Bromide	Medium	Permanent	Medium	Flexible	Linear	Side Chain	Weak
Polybrene	Low	Permanent	High	Flexible	Linear	Backbone	Moderate
p3-chloro-2-hydroxypropyl-methacryloxyethyldimethylammonium Cl	Medium	Permanent	High	Flexible	Linear	Side Chain	Moderate
Polydiallydimethylammonium Chloride	Medium	Permanent	High	Flexible	Cyclic	Side Chain	Weak
Polydiallydimethylammonium Chloride-*co*-Acrylamide	High	Permanent	Medium	Flexible	Cyclic	Side Chain	Moderate
Polydiallydimethylammonium Chloride-*co*-N-Isopropyl Acrylamide	Medium	Permanent	Medium	Flexible	Cyclic	Side Chain	Weak
Polydimethylaminoethyl Acrylate-*co*-Acrylamide (Quaternary)	High	Permanent	Medium	Flexible	Linear	Side Chain	Moderate
Polydimethylaminoethyl Methacrylate (Quaternized)	High	Permanent	High	Flexible	Linear	Side Chain	Weak
Polydimethylaminoethyl Methacrylate (Acryloxy)	High	Permanent	High	Flexible	Linear	Side Chain	Weak
Polydimethylamine Epichlorohydrin (Quaternized)	Low	Permanent	High	Flexible	Linear	Backbone	Weak

Table 3. (continued)

Polymer	Molecular weight[a]	Charge	Charge Density[b]	Chain Conformation	Backbone	Functional Group Attachment	Hydrogen Bonding
Polyethyleneimine	Low	Induced	High	Flexible	Branched	Backbone	Weak
Polyethyleneimine (Ethoxylated)	Low	Induced	Medium	Flexible	Branched	Backbone	Weak
Polyethyleneimine (hydroxylated, 50 kDa)	Low	Induced	High	Flexible	Branched	Backbone	Weak
Polyethyleneimine (hydroxylated, 70 kDa)	Low	Induced	High	Flexible	Branched	Backbone	Weak
Polyethyleneimine-Epichlorohydrin Modified	Low	Induced	Medium	Flexible	Linear	Backbone	Moderate
Polymethacryloxyethyl Trimethyl-ammonium Bromide	Medium	Permanent	High	Flexible	Linear	Side Chain	Moderate
Poly-2-hydroxy-3-methacryloxyethyl Trimethylammonium Chloride	Medium	Permanent	High	Flexible	Linear	Side Chain	Moderate
pHydroxyproplymethacryloxy Ethyl-dimethyl Ammonium Chloride	Medium	Permanent	High	Flexible	Linear	Side Chain	Moderate
Polyimidazoline (Quaternary)	Oligo	Permanent	High	Flexible	Linear/Cycl.	Backbone	Moderate
Polymethyl-Diethylaminoethyl-methacrylate-*co*- Acrylamide	High	Permanent	Medium	Flexible	Linear	Side Chain	Moderate
Polymethylene-*co*-Guanidine	Oligo	Induced	Medium	Flexible	Linear	Backbone	Weak
Poly-1-methyl-2-vinylpyridinium Bromide	High	Permanent	High	Flexible	Cyclic	Side Chain	Weak
Poly-1-methyl-4-vinylpyridinium Bromide	High	Permanent	High	Flexible	Cyclic	Side Chain	Weak
Polyvinylamine	Low-Medium	Induced	Medium	Flexible	Linear	Side Chain	Moderate
Polyvinylpyrrolidone-*co*-Dimethyl-aminoethylmethacrylate	Medium	Permanent	High	Flexible	Linear	Side Chain	Moderate

[a] Oligo (10^3) Low (10^4), Med. (10^5), High (10^6)
[b] Low (0-33 mol %), Medium (34-66 mol %), High (67-100 mol %).

2.4
Protocol for Polymer Evaluation

The evaluation of each individual polyelectrolyte and binary polyanion-polycation pairs has followed the protocol described in Fig. 1.

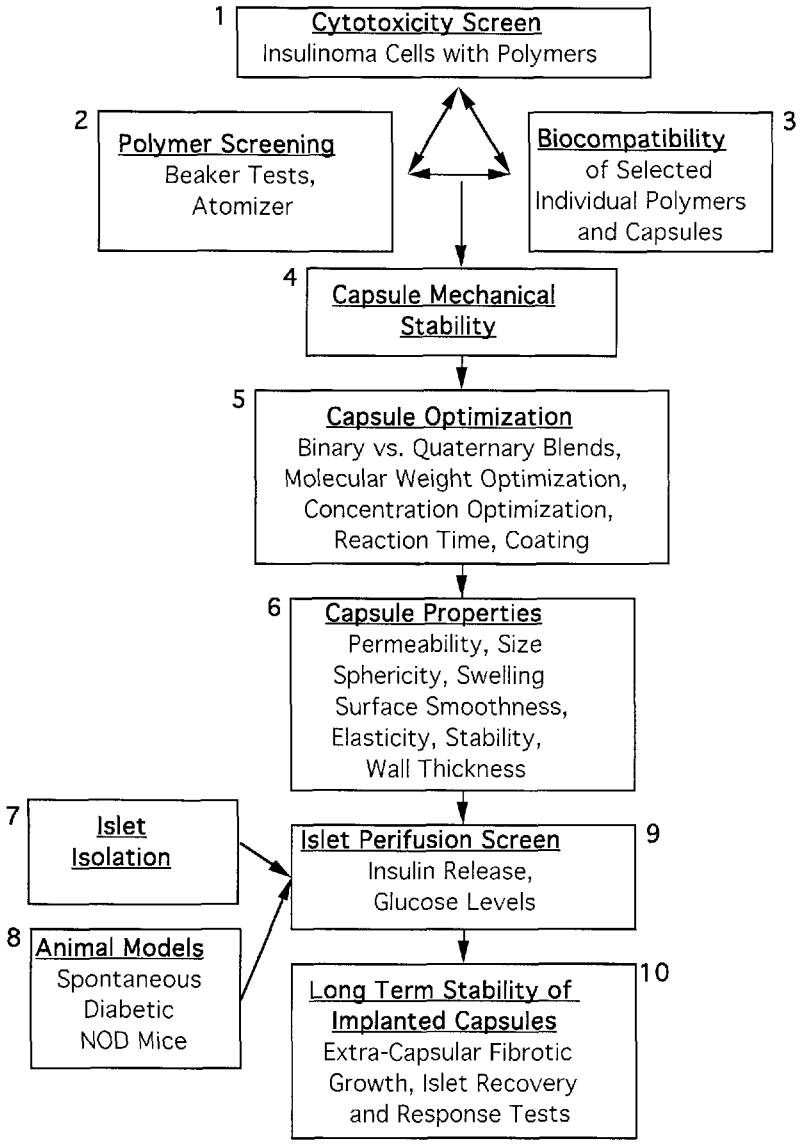

Fig. 1. Interrelationships between the various components of the polymer and capsule screening studies

- Step 1: Cytotoxicity. Each polymer was evaluated, at least twice, in blind experiments, according to their toxicity toward insulinoma cells. For many polymers alternative suppliers and molecular weights were also tested. In total 37 polyanions were obtained of which 23 were systematically evaluated. By comparison, 29 of the 36 polycations procured were systematically tested. The results of these experiments are provided in Table 4.
- Step 2: Polymer Screening
  (a) Various concentrations of a given polyanion were prepared at a pH of 5.5.
  (b) The polymers prepared in Step 2a were then extruded through a Pasteur pipette or syringe into a static beaker containing polycation solutions of various concentrations (pH=5.5). If 'n' concentrations of a polyanion were prepared in Step 2 and 'm' concentrations of the polycation were tested, this represented an nxm design for each binary pair of polycations and polyanions.
  (c) The testing in Step 2b was repeated at a pH of 7.0.
  (d) Based on the results of Steps 2–4, the reaction product of a given polyanion-polycation pair was visually characterized as a soluble complex, fine precipitate, fibrous precipitate, weak membrane or strong membrane. These results are shown in Table 5. Each entry in this table represents several experiments over a range of polymer concentrations. The overall ranking (soluble, precipitate, weak membrane, stable membrane) represents the best result observed for a given binary system for all polymer molecular weights, charge densities and suppliers which were evaluated. For example, polyacrylic acid was tested at various molecular weights, while polyacrylamide-*co*-dimethylaminoethylacrylate was evaluated at various charge densities. Similarly, carboxymethylcellulose was procured with various degrees of carboxy substitution.
  (e) The binary polyanion-polycations systems which yielded either weak or stable membranes, in Step 5, were then re-screened in the atomizer apparatus. In addition to the type of membrane produced the capsule swelling, shrinking and leakages were monitored as a function of time for 5 days. The membrane fusion, coagulant formation as well as visual metrics such as the capsule sphericity and opacity were also recorded. Given the hydrodynamic constraints of the piezoelectric atomizer, which placed a maximum viscosity of the droplet fluid, capsules were prepared under various concentrations and pH. The reaction time between the polyion droplet and the oppositely charged receiving bath was also varied. Finally, those systems which yielded stable membranes with polyanion interior to polycations were also tested in the reverse mode. Based on the screening in Step 2a–e, the best results for each system were recorded. For example, with the chitosan-tripolyphosphate system, a stable membrane was produced provided the cationic polymer was the inner material and the concentrations of the polycation and polyanion were 0.5–1.5 wt % and 3.0–6.0 wt % respectively, at pHs of 5.5 and 7.0. These results are shown in bold (symbol "SC") in Table 5. Table 6 lists the stability, transparency and surface smoothness of the best performing capsules from Table 5. These were selected as materials for further optimization.

Table 4. Cytotoxicity of polyelectrolytes on insulinoma cells

#	Weight percent polymer with supplier and grade	DNA content[a] (% of control)	Cell attachment[b] at 72 h	Overall rating[c]
	Naturally occurring and modified polyanions			
1a	1% Alginate (Sodium,) HV Keltone	23/57/53	1+/7+/7+	+
1a	1% Alginate (Sodium), HVCR Keltone	30/65/80	2+/6+/7+	++
1a	1% Alginate (Potasium) Keltone	40/35/30	2+/1+/1+	+/–
1b	1% Alginate (Sodium), LV Keltone	115/110/100	7+/7+/7+	++
1d	1% Alginate (Sodium), MV Sigma	55/55/55	+R/3+R/3+R	+/–
1e	1% Alginate (Sodium), UP LVG Pronova	48/88/130	5+/7+/7+	++
4b	1% Carboxymethyl Cellulose, 7MF Aqualon	110/135/110	5+/5+/5+	++
5	10% Carboxymethyl Dextran, Fluka	75/110/45	6+/4+R/+R	+/–
6a	0.7% Carrageenan-ι, 379 FMC	35/29/14	4+/3+/2+	+/–
6b	1% Carrageenan-κ, Sanofi	95/115/0	5+/5+/5+	++
6c	0.7% Carrageenan-λ, Fluka	70/80/70	5+/5+/3+	+
6d	1.5% Carrageenan-κ, FMC	120/60/0	7+/7+/R&F	+
7	2% Cellulose Sulfate, Janssen	35/5/0	3+/3+/3+	+/–
9	2% Chondroitin 6-Sulfate, Sigma	45/45/245	4+/7+/7+	++
10	10% Dextran Sulfate, Pharmacia	24/45/50	5+/5+/5+	+
11	0.6% Gellan/0.2% HMP, Kelco	60/56/30	4+/5+/2+R	+
14a	0.5% Hyaluronic Acid, Pronova	60/140/200	5+/6+/6+	++
14b	0.75% Hyaluronic Acid, Sigma	100/52/58	7+/7+/7+	++
15b	4% Pectin, SBI	74/70/87	5+/5+/5+	+
17a	0.15% Xanthan, Rhodigel, Vanderbilt	100/175/210	5+/7+/7+	++
17c	0.25% Xanthan, Keltrol, Kelco	80/120/240	6+/6+/6+	++

[a] A 72 hour incubation was used with 100, 200 and 500 µl of polymer added per ml of Phosphate Buffer Solution. The numbers indicate the DNA content relative to that observed in the control (PBS). A value over 100% signifies mitogenicity

[b] R&F: Round and Floating (dead cells), R: Rounded and Attached (healthy). Cell attachment is represented on a 1–7 scale. 3+R: Attached and growing with a small number of round cells, 7+: maximal cell attachment

[c] The last column is a cummulative metric of polymer-cell compatibility on an arbritrary – –, –, +/-, +, ++ scale.

Table 4. (continued)

#	Weight percent polymer with supplier and grade	DNA content[a] (% of control)	Cell attachment[b] at 72 h	Overall rating[c]
	Synthetic polyanions			
18	6% Pentasodiumripolyphosphate Hexahydrate, Sigma	0/0/0	R&F/R&F/R&F	– –
19	5% Polyacrylamide (Carboxy-Modified), Aldrich	85/90/50	5+/5+/3+	+
21	5 % Polyacrylamide-*co*-Acrylic Acid(30/70), 200 kDa, Polysciences	70/105/85	2+/2+R/2+R	+/–
22	2% Polyacrylamido-2-methyl-1-propanesulfonic acid, Aldrich	100/105/45	3+R/2+R/2+R	+
23a	4% Polyacrylic Acid, 60 kDa, Polysciences	0/0/0	R&F/R&F/R&F	– –
23a	10% Polyacrylic Acid, 140 kDa, Polysciences	25/0/0	1+R/R&F/R&F	–
23b	1% Polyacrylic Acid, 450 kDa, Polysciences	95/110/85	5+/5+/5+	+
23b	0.5% Polyacrylic Acid, 750 kDa, Aldrich	65/65/0	3+/3+/R&F	+/–
24	5% Polyglutamic Acid, Gelest	105/110/75	3+/3+/R&F	+
26	5% Polymaleic Anhydride, Polysciences	30/0/0	2+/R&F/R&F	–
27	2% Polymethacrylic Acid, Polysciences	55/50/25	3+R/+R/R&F	+/–
29	4.6% Polymethylvinylether maleic acid, Polysciences	30/0/0	1+R/R/R	–
30	2% Polystyrene Sulfonate, Polysciences	20/0/0	R&F/R&F/R&F	–
32	2% Polyvinylphosphonic Acid, Polysciences	65/0/0	R&F/R&F/R&F	–
34	10% Polyvinylsulfonic Acid (Sodium), Polysciences	50/0/0	4+/R/R&F	+/–
	Naturally occurring or biological polycations			
35a	1.4% Chitosan, LV Protan	25/55/45	R&F/R&F/R	+/–
35a	1.4% Chitosan, LV Protan (dialyzed)	80/15/25	3+/R&F/R&F	+/–
39	1% Lysozyme, Sigma	36/64/92	3+/3+/5+	+

[a] A 72 hour incubation was used with 100, 200 and 500 µl of polymer added per ml of Phosphate Buffer Solution. The numbers indicate the DNA content relative to that observed in the control (PBS). A value over 100% signifies mitogenicity

[b] R&F: Round and Floating (dead cells), R: Rounded and Attached (healthy). Cell attachment is represented on a 1–7 scale. 3+R: Attached and growing with a small number of round cells, 7+: maximal cell attachment

[c] The last column is a cummulative metric of polymer-cell compatibility on an arbritrary – –, –, +/-, +, ++ scale.

Table 4. (continued)

#	Weight percent polymer with supplier and grade	DNA content[a] (% of control)	Cell attachment[b] at 72 h	Overall rating[c]
40	0.25% Poly-L-Lysine, 55 kDa, Sigma	15/30/100	R/R/R	+/–
42a	3.5% Protamine Sulfate, Grade III, Sigma	350/200/25	4+/6+/5+	+
42b	1% Protamine Sulfate, Fluka	42/0/0	3+/R&F/R&F	+/–
	Synthetic Polycations			
43b	5% Polyamide (Cationic), 5087, Callaway	0/0/0	R&F/R&F/R&F	– –
43b	3% Polyamine (Cationic), 4030, Callaway	0/0/0	R&F/R&F/R&F	– –
43b	5% Polyacrylamide (Cationic), 5087, Callaway	0/0/0	R&F/R&F/R&F	– –
45	5% Polyallylamine, 57 kDa , Polysciences	25/0/0	R&F/R&F/R&F	– –
48	0.2% Polyamine (Quaternized), B50, CPS	0/0/0	R/R/R&F	–
49	1-5% Polybrene, Sigma	0/0/0	R&F/R&F/R&F	– –
51	5% Poly-3-chloro-2-hydroxypropylmethacryloxyethyl dimethylammoniumchloride, Polysciences	0/0/0	R/R/R	–
52b	1% Polydiallyldimethylammonium Chloride, Polysciences	0/0/0	R&F/R&F/R&F	– –
53	2% Polydiallyldimethylammonium Chloride-*co*-Acrylamide, C3204 CPS	0/0/0	R/R/R	–
56	1-4% Polydimethylamine Epichlorohydrin (Quaternized), SPP	0/0/0	R/R/R	–
57a	0.05% Polydimethylaminoethyl Acrylate-*co*-Acrylamide, 1158, Betz	130/150/150	6+/6+/2+R	++
59	5% Polydimethylaminoethyl Methacrylate (Quaternized), Polysciences	0/0/0	R/R/R	–
61	5% Polyethyleneimine, BASF	0/0/0	R/R/R	–

[a] A 72 hour incubation was used with 100, 200 and 500 µl of polymer added per ml of Phosphate Buffer Solution. The numbers indicate the DNA content relative to that observed in the control (PBS). A value over 100% signifies mitogenicity

[b] R&F: Round and Floating (dead cells), R: Rounded and Attached (healthy). Cell attachment is represented on a 1–7 scale. 3+R: Attached and growing with a small number of round cells, 7+: maximal cell attachment

[c] The last column is a cummulative metric of polymer-cell compatibility on an arbritrary – –, –, +/-, +, ++ scale.

Table 4. (continued)

#	Weight percent polymer with supplier and grade	DNA content[a] (% of control)	Cell attachment[b] at 72 h	Overall rating[c]
62	5% Polyethyleneimine-Epichlorohydrin, SPP	0/0/0	R/R/R	–
63	5% Polyethyleneimine (hydroxyethylated, 50 kDa), Polysciences	0/0/0	R&F/R&F/R&F	– –
64	3.5% Polyethyleneimine, ethoxylated, 70 kDa , SPP	0/0/0	R&F/R&F/R&F	– –
69	5% Polymethacryloxyethyltrimethylammonium Bromide, Polysciences	0/0/0	R/R/R	–
69	5% Polymethyloxyethyltrimethylammonium Chloride, Polysciences	0/0/0	R/R/R	–
72	2% Poly-1-methyl-2-vinylpyridinium Bromide, Polysciences	0/0/0	R&F/R&F/R&F	– –
73	2% Poly-1-methyl-4-vinylpyridinium Bromide, Polysciences	0/0/0	R&F/R&F/R&F	– –
74	0.1% Polymethylene-*co*-Guanidine, Aldrich	30/0/0	R/R/R	–
75	1-5% Polyvinylamine, 70 kDa , Air Products	20/20/20	R/R/R	–
75	4% Polyvinylamine, 220 kDa , Air Products	0/0/0	R/R&F/R&F	–
77	5% Poly-4-vinylbenzyltrimethylammonium Chloride, SPP	0/0/0	R&F/R&F/R&F	– –
78	15% Poly-4-vinylbenzyltrimethylammonium Chloride, Polysciences	0/0/0	R&F/R&F/R&F	– –

[a] A 72 hour incubation was used with 100, 200 and 500 µl of polymer added per ml of Phosphate Buffer Solution. The numbers indicate the DNA content relative to that observed in the control (PBS). A value over 100% signifies mitogenicity

[b] R&F: Round and Floating (dead cells), R: Rounded and Attached (healthy). Cell attachment is represented on a 1–7 scale. 3+R: Attached and growing with a small number of round cells, 7+: maximal cell attachment

[c] The last column is a cummulative metric of polymer-cell compatibility on an arbritrary – –, –, +/-, +, ++ scale.

Table 5. Binary screening of polyanions and polycations

Polymer	Chitosan	Diethylaminoethyl Dextran	Gelatin A	Glycol Chitosan	Hydroxymethylcellulose-Q	Lysozyme	Polyacrylamide-Cationic	Polyacrylamide-Cationic #2	Polyacrylamide-MAOETMAC	Polyallylamine	Polyamide-Q	Polyamine	Polyamine-Q	Polybrene	PolyBA-MAOE-TMAC	PolyCHP-MAOEDMAC	PolyDADMAC	PolyDADMAC-Acrylamide	PolyDADMAC-NIPAM	PolyDMAEA-Acrylamide
Naturally occurring polyanions																				
Alginate (Sodium)	**SC**	wm	wm	SM	pt	–	wm	wm	pt	wm	wm	wm	pt	pt	pt	pt	pt	wm	pt	wm
Alginate (Propylene Glycol Modified)	SM	pt	pt	SM	pt	–	pt	wm	pt	wm	sol	wm	pt	–	wm	pt	pt	pt	pt	wm
Carboxymethyl Amylose	pt	pt	pt	pt	pt	–	pt	wm	pt	wm	pt	pt	pt	–	pt	pt	wm	wm	pt	wm
Carboxymethyl Cellulose	SM	wm	wm	pt	wm	–	SM	pt	pt	**SC**	wm	wm	**SC**	pt	pt	pt	wm	wm	pt	wm
Carboxymethyl Dextran	wm	pt	wm	wm	pt	–	pt	wm	pt	pt	sol	wm	pt	–	pt	pt	wm	wm	pt	wm
Carrageenan (λ)	wm	wm	SM	wm	pt	sol	wm	wm	pt	wm	wm	wm	**SC**	–	wm	**SC**	wm	wm	wm	pt
Cellulose Sulfate	SM	wm	wm	SM	pt	–	wm	wm	pt	**SC**	wm	wm	**SC**	–	pt	wm	SM	wm	SM	SM
Chondroitin 4-Sulfate	wm	sol	pt	pt	pt	–	wm	wm	pt	pt	sol	pt	pt	–	pt	pt	wm	pt	pt	wm
Chondroitin 6-Sulfate	wm	pt	pt	pt	pt	–	wm	wm	pt	pt	wm	pt	pt	wm	pt	pt	wm	wm	pt	wm
Dextran Sulfate	wm	pt	wm	pt	pt	–	wm	SM	pt	sol	pt	wm	SM	–	pt	pt	wm	SM	pt	wm
Gellan Gum	wm	wm	SM	wm	wm	–	wm	wm	wm	SM	**SC**	SM	**SC**	SM	SM	SM	SM	wm	wm	wm
Gelatin A (pH = 9.0)	sol	sol	sol	sol	sol	–	sol	wm	sol	sol	sol	sol	sol	–	sol	wm	pt	sol	SM	wm
Gelatin B (pH = 6.5)	sol	sol	sol	wm	sol	–	sol	wm	wm	wm	sol	sol	sol	–	wm	wm	wm	sol	SM	pt
Gum Arabic	wm	sol	sol	sol	pt	–	wm	pt	sol	sol	sol	sol	sol	–	pt	sol	pt	pt	pt	SM
Heparin	wm	pt	pt	pt	pt	–	pt	wm	pt	pt	sol	wm	pt	–	pt	wm	wm	wm	pt	wm
Hyaluronic Acid	wm	SM	wm	wm	SM	–	wm	wm	pt	wm	wm	SM	wm	–	wm	SM	SM	SM	pt	wm
Pectin (low esterification)	–	–	–	–	–	–	–	pt	–	SM	pt	–	–	wm	–	–	wm	pt	–	–
Polygalacturonic Acid	wm	wm	wm	wm	wm	sol	wm	wm	pt	wm	wm	wm	wm	wm	pt	pt	wm	wm	pt	wm
Xanthan	wm	wm	wm	wm	wm	–	wm	wm	wm	**SC**	SM	SM	**SC**	SM	SM	**SC**	SM	**SC**	SM	SM

Table 5. (continued)

Synthetic Polyanions																				
Pentasodiumtripolyphosphate Hexahydrate	***SC***	pt	sol	pt	sol	–	wm	sol	sol	pt	sol	sol	sol	–	pt	pt	sol	sol	sol	pt
Polyacrylamide (90% Carboxy-Modified)	wm	wm	pt	pt	pt	–	wm	wm	pt	wm	pt	wm	pt	–	pt	pt	pt	wm	pt	wm
Polyacrylamide (70% Carboxy-Modified)	wm	pt	sol	pt	pt	–	sol	wm	pt	wm	sol	sol	wm	–	wm	wm	wm	sol	sol	sol
Polyacrylamide-*co*-Acrylic Acid	wm	sol	pt	pt	wm	–	pt	wm	pt	pt	pt	pt	pt	–	pt	pt	wm	wm	pt	wm
Polyacrylamide (methylpropanesulfonicacid)	wm	wm	SM	pt	wm	–	wm	wm	pt	wm	pt	pt	pt	–	wm	wm	wm	wm	pt	SM
Polyacrylic Acid	SM	wm	wm	SM	pt	–	wm	SM	SM	SM	wm	SM	wm	–	SM	SM	SM	SM	SM	SM
Polyglutamic Acid	***SC***	sol	sol	wm	pt	–	pt	wm	pt	pt	sol	sol	sol	–	pt	pt	pt	pt	pt	wm
Polymaleic Acid	SM	pt	sol	pt	pt	–	wm	pt	pt	pt	sol	sol	sol	–	sol	sol	pt	pt	sol	wm
Polymaleic Anhydride	SM	sol	wm	sol	pt	–	pt	wm	pt	wm	sol	sol	sol	–	pt	pt	pt	wm	sol	wm
Polymethacrylic Acid	wm	pt	pt	pt	pt	–	pt	pt	pt	pt	pt	wm	pt	–	pt	pt	pt	pt	pt	wm
Polymethylvinylether Maleic Acid	wm	sol	pt	pt	pt	–	pt	wm	pt	pt	pt	wm	pt	–	pt	pt	pt	SM	pt	wm
Polymethylvinylether Maleic Anhydride	wm	sol	wm	sol	wm	–	wm	wm	pt	pt	wm	wm	pt	–	pt	wm	wm	wm	pt	wm
Polystyrene Sulfonate	***SC***	pt	wm	pt	pt	–	pt	wm	pt	pt	pt	wm	pt	–	wm	wm	wm	wm	pt	pt
Polyvinylphosphate	pt	sol	sol	pt	sol	–	wm	pt	pt	sol	sol	sol	sol	–	pt	sol	sol	pt	pt	SM
Polyvinylphosphonic Acid	SM	pt	pt	pt	pt	–	wm	pt	sol	pt	pt	pt	pt	–	pt	pt	pt	pt	pt	wm
Polyvinylsulfonic Acid (Sodium)	SM	pt	wm	pt	pt	–	wm	pt	pt	pt	wm	wm	pt	–	pt	pt	pt	wm	pt	wm

Acronyms

Polymers: BA : Butylacrylate, CHP : 3-chloro-2-hydroxypropyl, DADMAC : Diallyldimethylammonium chloride, DMAEA : Dimethylaminoethylacrylate, DMAEM : Dimethylaminoethylmethacrylate, EM : Epichlorohydrin Modified, HMAOETMAC : Hydroxymethacryloxyethyltrimethylammoniumc chloride, MAOEDMAC : Methacryloxyethyldimethylammonium chloride, MAOETMAC : Methacryloxyethyltrimethylammonium chloride, MDMAEM : Methyldimethylaminoethylacrylate, M2VP : 1-methyl-2-vinylpyridinium bromide, M4VP : 1-methyl-4-vinylpyridinium bromide, NIPAM : *N*-isopropylacrylamide, PEI : Polyethyleneimine, Q : Quaternary, VBTMAC : Vinylbenzyltrimethylammonium chlroide, VP : Vinylpyridinium.

Membranes: pt : precipitate, sol : soluble complex, **SC** : stable capsule with a polyanion interior to a polycation, ***SC*** : stable capsule with a polycation interior to a polyanion, SM : stable membrane but a capsule with structural integrity could not be produced, wm : weak membrane.

Table 5. (continued)

Polymer	PolyDMAEA-Acrylamide	Polydimethylacrylate-EM-Q	PolyDMAEM	PolyDMAEM-Acrylamide	PEI	PEI-Epichlorohydrin	PEI-Ethoxylated	PEI-Hydroxyethylated	PolyHMAOETMAC	Polyimidozoline	Poly-L-Lysine	PolyMAOETAC	PolyMDMAEA-Acrylamide	Polymethylene-co-guanidine	PolyM2VP	PolyM4VP	Polyvinylamine	PolyVBTMAC	PolyVP-DMAEM-Q	Protamine
Naturally occurring polyanions																				
Alginate (Sodium)	pt	pt	pt	wm	sol	wm	–	wm	pt	pt	wm	pt	wm	**SC**	pt	pt	wm	pt	pt	pt
Alginate (Propylene Glycol Modified)	pt	pt	pt	wm	pt	wm	–	wm	pt	pt	wm	pt	wm	wm	pt	pt	wm	–	pt	–
Carboxymethyl Amylose	pt	pt	wm	wm	pt	wm	–	pt	wm	pt	pt	pt	wm	pt	pt	pt	wm	wm	wm	–
Carboxymethyl Cellulose	pt	pt	pt	wm	sol	wm	–	wm	pt	pt	pt	pt	wm	wm	pt	pt	**SC**	wm	pt	–
Carboxymethyl Dextran	pt	pt	pt	wm	pt	pt	–	pt	pt	pt	pt	pt	wm	pt	pt	pt	wm	wm	wm	–
Carrageenan (λ)	wm	**SC**	**SC**	wm	sol	wm	SM	SM	SM	wm	wm	**SC**	wm	SM	**SC**	**SC**	**SC**	SM	wm	SM
Cellulose Sulfate	SM	**SC**	wm	wm	sol	SM	**SC**	SM	wm	wm	pt	wm	wm	SM	**SC**	**SC**	SM	SM	pt	wm
Chondroitin 4-Sulfate	pt	pt	pt	wm	pt	pt	–	pt	pt	pt	pt	pt	wm	pt	pt	pt	–	–	sol	–
Chondroitin 6-Sulfate	pt	pt	pt	wm	pt	pt	–	pt	pt	pt	pt	pt	wm	pt	pt	pt	**SC**	wm	wm	–
Dextran Sulfate	pt	pt	sol	wm	sol	pt	–	pt	pt	pt	pt	pt	wm	SM	wm	wm	**SC**	pt	pt	–
Gellan Gum	SM	**SC**	SM	wm	wm	SM	**SC**	**SC**	SM	SM	**SC**	**SC**	wm	SM	SM	SM	**SC**	SM	wm	SM
Gelatin A (pH = 9.0)	sol	sol	sol	wm	sol	wm	–	sol	sol	pt	sol	sol	wm	pt	sol	sol	wm	–	sol	–
Gelatin B (pH = 6.5)	wm	sol	sol	wm	sol	pt	–	sol	wm	sol	pt	wm	pt	sol	wm	pt	wm	–	wm	–
Gum Arabic	sol	sol	pt	wm	sol	sol	–	sol	sol	sol	sol	sol	wm	sol	sol	sol	–	–	pt	–
Heparin	pt	pt	pt	wm	wm	wm	–	pt	pt	pt	sol	pt	sol	wm	pt	pt	wm	–	sol	–
Hyaluronic Acid	SM	pt	pt	wm	pt	SM	–	wm	wm	wm	wm	SM	wm	wm	wm	wm	SM	wm	wm	–
Pectin (low esterification)	–	wm	–	–	–	–	–	wm	–	wm	–	–	–	SM	–	–	SM	–	–	–
Polygalacturonic Acid	pt	pt	wm	wm	wm	wm	wm	wm	pt	pt	pt	pt	pt	pt	wm	wm	wm	–	pt	–
Xanthan	SM	wm	**SC**	SM	sol	**SC**	SM	**SC**	wm	SM	**SC**	SM	SM	**SC**	**SC**	**SC**	**SC**	wm	wm	SM

Table 5. (continued)

Synthetic Polyanions																				
Pentasodiumtripolyphosphate Hexahydrate	–	sol	pt	wm	pt	sol	–	sol	sol	sol	pt	pt	sol	pt	wm	sol	**SC**	sol	sol	–
Polyacrylamide (90% Carboxy-Modified)	sol	pt	pt	wm	pt	wm	–	pt	pt	pt	pt	pt	wm	pt	pt	pt	wm	wm	pt	–
Polyacrylamide (70% Carboxy-Modified)	pt	pt	wm	wm	pt	sol	–	sol	wm	pt	wm	wm	pt	sol	wm	wm	wm	sol	wm	–
Polyacrylamide-*co*-Acrylic Acid	pt	pt	pt	wm	pt	pt	–	pt	pt	pt	pt	pt	pt	pt	pt	pt	wm	sol	wm	–
Polyacrylamide (methylpropanesulfonicacid)	pt	pt	pt	wm	pt	sol	–	pt	wm	pt	pt	SM	wm	SM	SM	SM	wm	wm	wm	–
Polyacrylic Acid	pt	SM	wm	SM	pt	**SC**	wm	SM	pt	SM	**SC**	SM	wm	SM	SM	SM	wm	wm	SM	–
Polyglutamic Acid	SM	pt	pt	wm	sol	wm	–	pt	pt	pt	pt	pt	pt	pt	pt	pt	wm	pt	pt	–
Polymaleic Acid	sol	pt	sol	sol	pt	sol	–	pt	sol	pt	pt	sol	wm	pt	wm	sol	wm	sol	sol	–
Polymaleic Anhydride	pt	sol	pt	wm	pt	pt	–	sol	pt	sol	pt	pt	pt	pt	sol	sol	wm	wm	pt	–
Polymethacrylic Acid	wm	pt	pt	wm	pt	pt	–	pt	pt	pt	pt	pt	wm	pt	pt	pt	wm	pt	pt	–
Polymethylvinylether Maleic Acid	pt	pt	pt	wm	pt	pt	–	pt	pt	pt	pt	pt	wm	pt	pt	pt	wm	wm	wm	–
Polymethylvinylether Maleic Anhydride	pt	pt	pt	wm	pt	pt	–	pt	pt	pt	pt	pt	wm	pt	pt	pt	wm	wm	pt	–
Polystyrene Sulfonate	pt	pt	pt	pt	sol	pt	–	pt	pt	pt	pt	wm	wm	pt	pt	pt	wm	pt	wm	–
Polyvinylphosphate	pt	sol	sol	wm	pt	sol	–	sol	sol	sol	pt	sol	wm	sol	sol	sol	–	–	pt	–
Polyvinylphosphonic Acid	sol	pt	sol	wm	pt	pt	–	pt	pt	pt	pt	pt	wm	pt	pt	pt	wm	pt	pt	–
Polyvinylsulfonic Acid (Sodium)	pt	pt	pt	wm	pt	pt	–	pt	pt	pt	pt	pt	wm	pt	pt	pt	wm	pt	wm	–

Acronyms

Polymers: BA : Butylacrylate, CHP : 3-chloro-2-hydroxypropyl, DADMAC : Diallyldimethylammonium chloride, DMAEA : Dimethylaminoethylacrylate, DMAEM : Dimethylaminoethylmethacrylate, EM : Epichlorohydrin Modified, HMAOETMAC : Hydroxymethacryloxyethyltrimethylammoniumc chloride, MAOEDMAC : Methacryloxyethyldimethylammonium chloride, MAOETMAC : Methacryloxyethyltrimethylammonium chloride, MDMAEM : Methyldimethylaminoethylacrylate, M2VP : 1-methyl-2-vinylpyridinium bromide, M4VP : 1-methyl-4-vinylpyridinium bromide, NIPAM : *N*-isopropylacrylamide, PEI : Polyethyleneimine, Q : Quaternary, VBTMAC : Vinylbenzyltrimethylammonium chlroide, VP : Vinylpyridinium.

Membranes: pt : precipitate, sol : soluble complex, **SC** : stable capsule with a polyanion interior to a polycation, ***SC*** : stable capsule with a polycation interior to a polyanion, SM : stable membrane but a capsule with structural integrity could not be produced, wm : weak membrane.

Table 6. Stability, swelling and transparency of selected capsules in water and phosphate buffer solution

Polymer	Media	Alginate (Sodium)	Cellulose Sulfate	Carboxy-methyl Cellulose	Xanthan	Carrageenan (λ)	Gellan Gum	Poly Glutamic Acid
Naturally occurring or biological polycations								
Chitosan	PBS Water	S&S,T,L S&S,T,L	**S&S,T,SiM** S&S,T	–	–	–	–	**S&S,NT,R** S&S,NT
Polylysine	PBS Water	–	–	–	**S&S,ST** S&S,ST,L	–	S&S,T,L PC,T	–
Synthetic polycations								
Polyethyleneimine (ethoxylated)	PBS Water	–	PC,NT PC,NT	–	S&S,ST S&S,ST	PC,L,NT,S PC,L,NT,S	**S&S,T** S&S,T	–
Polyethyleneimine (Epichlorohydrin Modified)	PBS Water	–	S&S,T,L S&S,T	–	S&S,ST S&S,ST	–	PC,T PC,T	–
Polydiallyldimethylammonium Chloride-*co*-Acrylamide	PBS Water	–	–	S&S,NT,L S&S,NT,L	S&S,ST S&S,ST	–	–	–
Polyallylamine	PBS Water	S&S,T,L S&S,T,L	S&S,T,L S&S,T,L	**S&S,T,S,SiM** S&S,T,S	**S&S,ST** S&S,ST	–	**S&S,T** S&S,T	–
Polyvinylamine	PBS Water	S&S,T S&S,T	**S&S,T,SiM** S&S,T,L	**S&S,T,S,SiM** S&S,T,S	PC,NT S&S,NT	–	**S&S,T** S&S,T	–
Polydimethylaminoethyl Methacrylate (Quaternized)	PBS Water	–	–	–	S&S,ST S&S,ST	**S&S,ST** S&S,ST	PC,T PC,T	–
Polymethacryoxyethyl Trimethylammonium Bromide	PBS Water	–	S&S,T,L S&S,T,L	–	S&S,ST,L S&S,ST,L	**S&S,T** S&S,T	–	–
Poly-3-chloro-2-hydroxypropyl methacryloxyethyl dimethylammonium Chloride	PBS Water	–	**S&S,T,SiM** S&S,T,L	–	S&S,ST S&S,NT	**S&S,T** S&S,T	PC,ST PC,ST	–

Table 6. (continued)

Polymer	Media	Alginate (Sodium)	Cellulose Sulfate	Carboxy-methyl Cellulose	Xanthan	Carrageenan (λ)	Gellan Gum	Poly Glutamic Acid
Polyamine (Quaternized)	PBS Water	–	S&S,ST,S,SiM S&S,ST,L	S&S,NT,L S&S,NT,L	S&S,ST PC,ST	S&S,NT,L S&S,NT	PC,ST PC,ST	–
Polyamide	PBS Water	–	–	–	–	–	S&S,ST S&S,ST	–
Polydimethylamino-*co*-Epichlorohydrin (Quaternized)	PBS Water	–	**S&S,ST,SiM** S&S,L,NT	–	–	S&S,NT,S S&S,NT	S&S,ST S&S,ST	–
Polymethylene-*co*-Guanidine	PBS Water	**S&S,ST,SiM** S&S,NT,L	**S&S,ST,SiM** S&S,NT	–	**S&S,NT** S&S,NT	PC,NT PC,NT	PC,NT S&S,ST	–
Polymethyl-2-vinylpyridinium Bromide	PBS Water	–	**S&S,T,SiM** S&S,T	–	PC,ST S&S,ST	PC,T S&S,T	PC,ST S&S,ST	–
Polymethyl-4-vinylpyridinium Bromide	PBS Water	–	**S&S,T,SiM** S&S,T	–	PC,ST S&S,ST	PC,T S&S,T	PC,NT S&S,ST	–

L : Leaking, NT : Non-Transparent, PC : Partially Collapsed, R : Reversed (Cation Interior), S : Sticky, ST : Semi-Transparent, SiM : Stable in Media, S&S : Smooth and Swollen, T : Transparent

Table 6. (continued)

Polymer	Media	Polymaleic Anhydride	Poly Acrylic Acid	Polyvinyl Phosphonic Acid	Polyvinyl Sulfonic Acid	Polystyrene Sulfonate	Tripoly Phosphate
Naturally occurring or biological polycations							
Chitosan	PBS Water	S&S,T PC,T	–	S&S,ST,PC S&S,ST,PC	**S&S,NT,R** S&S,NT	**S&S,T,SiM,R** S&S,T,L	**S&S,NT,SiM,R** S&S,NT
Polylysine	PBS Water	–	–	–	–	–	–
Synthetic polycations							
Polyallylamine	PBS Water	–	–	–	–	–	–
Polyamine (Quaternized)	PBS Water	–	–	–	–	–	–
Polyamide	PBS Water	–	–	–	–	–	–
Poly-3-chloro-2-hydroxypropyl methacryloxyethyl dimethylammonium Chloride	PBS Water	–	–	–	–	–	–
Polydiallyldimethylammonium Chloride-*co*-Acrylamide	PBS Water	–	–	–	–	–	–
Polydimethylamino-*co*-Epichlorohydrin (Quaternized)	PBS Water	–	–	–	–	–	–
Polydimethylaminoethyl Methacrylate (Quaternized)	PBS Water	–	–	–	–	–	–
Polyethyleneimine (ethoxylated)	PBS Water	–	–	–	–	–	–

Table 6. (continued)

Polymer	Media	Polymaleic Anhydride	Poly Acrylic Acid	Polyvinyl Phosphonic Acid	Polyvinyl Sulfonic Acid	Polystyrene Sulfonate	Tripoly Phosphate
Polyethyleneimine-Epichlorohydrin	PBS Water	–	S&S,ST,L S&S,ST,L	–	–	–	–
Polymethacryoxyethyl Trimethylammonium Bromide	PBS Water	–	–	–	–	–	–
Polymethylene-*co*-Guanidine	PBS Water	–	–	–	–	–	–
Polymethyl-2-vinylpyridinium Bromide	PBS Water	–	–	–	–	–	–
Polymethyl-4-vinylpyridinium Bromide	PBS Water	–	–	–	–	–	–
Polyvinylamine	PBS Water	–	–	–	–	–	**S&S,NT,R** S&S,NT

L : Leaking, NT : Non-Transparent, PC : Partially Collapsed, R : Reversed (Cation Interior), S : Sticky, ST : Semi-Transparent, SiM : Stable in Media, S&S : Smooth and Swollen, T : Transparent

- Step 3: Biocompatibility. The biocompatibility of selected polymers, identified in Steps 1 and 2, were evaluated by implanting flat membranes into a C57/B16 mouse (Jackson Labs, Bar Harbor, ME). The membranes and capsules were implanted at various internal sites or in the back tissue under the skin. The results of these tests are not reported herein and will be discussed in a subsequent publication.* They do, however, have important implications as to the ultimate selection of a polymeric system.
- Step 4: Capsule Mechanical Stability. The mechanical stability of the membranes was assessed semi-quantitatively by applying a compressional force via a micrometer. While this method is not precise, it did permit us to assess if the capsules could withstand deformations and if they ruptured in a controlled or catastrophic manner. Another test which was selectively employed was to place capsules between microscope slides and measure the force required to compromise the integrity of the membrane. These tests measured the resistance of the weakest point of the membrane. For certain capsules a needle was used to probe the breaking strength of a local region of the membrane.
- Steps 5 and 6 involved the characterization and optimization of multicomponent capsules based on blends containing up to four polyelectrolytes, often in the presence of a gelling agent or surface coating. While these results are part of the overall objective of this project, they are beyond the scope of this paper and will be reported elsewhere [61, 62].
- Steps 7–10 involved the selection of animal models, islet isolation and the testing of the polymer microcapsules as bioartificial organs. This has been discussed elsewhere [61, 62].

2.5 Capsule Treatment

Each polyion pair which yielded a stable membrane was removed from the receiving bath and treated. In general, quintuple washings with an excess (50 ml) of PBS were required to remove all traces of the polymeric reagents. The PBS also simulated the osmotic pressure which the capsule, and mammalian cells, would encounter in vivo. For several polyanion-polycation systems the membranes which were produced were not sufficiently strong to survive the rinsings. Leaky membranes and the complete collapse of the capsule were two common failures.

2.6 Beaker Screening Tests

Two methods of capsule formation were employed: static beaker tests and atomizer screenings. In the beaker tests, which comprised the first phase of the screening (Step 2 of Fig. 1), a small volume of "inner" polymer solution was extruded from a Pasteur pipette as a droplet (nominally 2–3 mm) into a receiv-

* Permeability measurements have also been described elsewhere.

ing bath which contained an excess volume (20–30 ml in a 50-ml beaker) of the "outer" polymer solution. The resulting microcapsule generally contained a polyanion core and a thin film exterior membrane, though inverse systems were also tested with polycations interior to the capsules. The anionic polymers were preferred, a priori, as inner materials since many living cells contain polyanions in their extracellular matrix. The cytotoxicity studies, which will be presented herein, will also indicate the superiority of polyanions as cell suspending solutions. The beaker screening procedure was as follows: 5–10 droplets of the inner polymer were allowed to fall a distance of 3 cm into the receiving bath. The type of polyelectrolyte coacervate formed (soluble complex, precipitate, weak membrane, stable membrane) was observed immediately after the contacting of the two oppositely charged solutions and then again after 15–30 min. While the distinction between soluble complex, precipitate and membrane is unambiguous, a "stable" membrane was defined as one which remained intact following a gentle hand agitation of the beaker. In contrast "weak" membranes and capsules were broken upon the application of hydrodynamic forces. The type of precipitate (microprecipitate, fibrous/coagulated, fibrous gel) and the capsule surface characteristics (smooth/wrinkled, shrinking/swelling, leaking/leaching of the inner polymer) were also recorded. Capsules were observed to float on the surface of the receiving bath or to sediment depending on the densities of the polymer solutions. Additionally, some capsules adhered to the collecting reservoir employed (glass or plastic). All polyanion-polycation pairs which yielded "stable" membranes with the polyanion interior to the polycation were subsequently tested in their reverse mode (polycations droplets dispersed in a polyanion receiving bath). The ultimate stability test, and an indication of the sensitivity of the coacervate to ionic strength, was the ability of the microcapsules membrane to withstand a PBS wash.

2.7
Atomizer Screening

The atomizer screening (Fig. 2) was carried out using a droplet generator which consisted of a small nozzle (0.2–0.5 mm) attached to a piezoelectric collar. This produced a perturbation with a controlled frequency and amplitude which resulted in discrete droplet formation downstream from the jet exit [63]. The liquid jet velocity was regulated and varied between 1 and 3 m/s for different experiments. The piezoelectric collar (Krohnhite Corporation, Avon, MA) was driven by a function generator (3325B Synthesizer, Hewlett-Packard, Palo Alto, CA) with frequencies up to 3 kHz. A high current DC power amplifier (GFA-555, Adcom Technologies, East Brunswick, NJ) was used as a power source. Each droplet was charged by employing an electrode system coupled to the power source. This had the effect of creating a spray of approximately 500-μm droplets which prevented capsule aggregation or fusion in the receiving bath. Since this approach combined droplet atomization and impact conditions it is referred to herein as the "atomizer/impact" method.

For highly viscoelastic polymer solutions the piezoelectric device was substituted with an air stripping apparatus (Fig. 3). Using this technique a concentric two-nozzle system has been constructed. The inner nozzle (0.2–0.5 mm ID) was used to extrude the core fluid while the outer nozzle, through which air passes at a controlled rate, was used to strip and atomize the liquid stream into small droplets. A liquid flow rate in the range of 1–10 ml/min was employed with a corresponding air flowrate set between 100 and 1000 times the liquid flow. The air and liquid flow rates were controlled using pressure regulators (Type 700, Control Air, Amherst, NH) and needle valves (Whitey Co., Highland Heights, OH). Polyelectrolyte droplets in the 400–800 μm size were collected in 10-ml beakers containing an oppositely charged polyion solution.

A limited number of polyanion-polycation systems were tested using a droplet/falling annulus method (Fig. 4). This technique, which has been described elsewhere [64] reduces the net impact velocity between the droplet with the oppositely charged counterion fluid. A stream of droplets was directed into a collapsing annular liquid sheet. By matching the velocities of the droplet and sheets, the impact conditions can be moderated. It has been shown to produce monodisperse spherical capsules, though it requires several days of calibration for each new system and is obviously not practical for a massive screening such as was carried out herein.

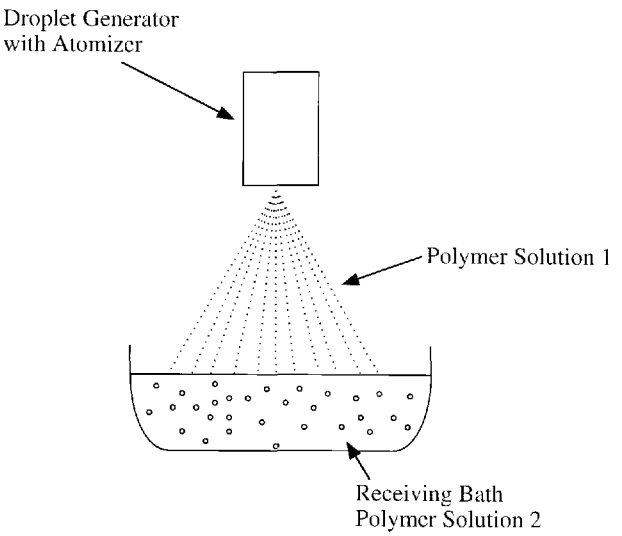

Fig. 2. Schematic of the droplet atomizer showing the piezoelectric collar and experimental setup

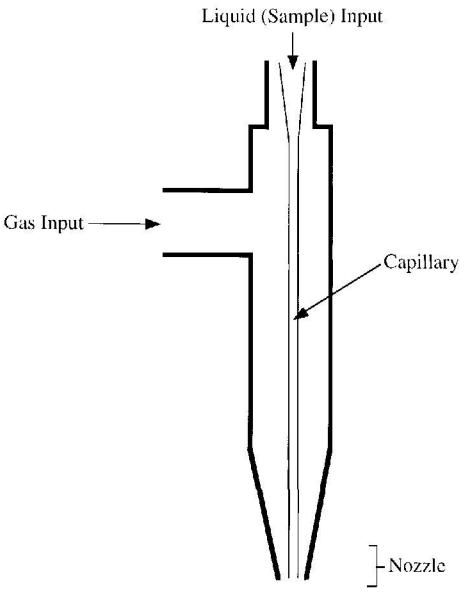

Fig. 3. Schematic of the droplet atomizer/air-stripping device

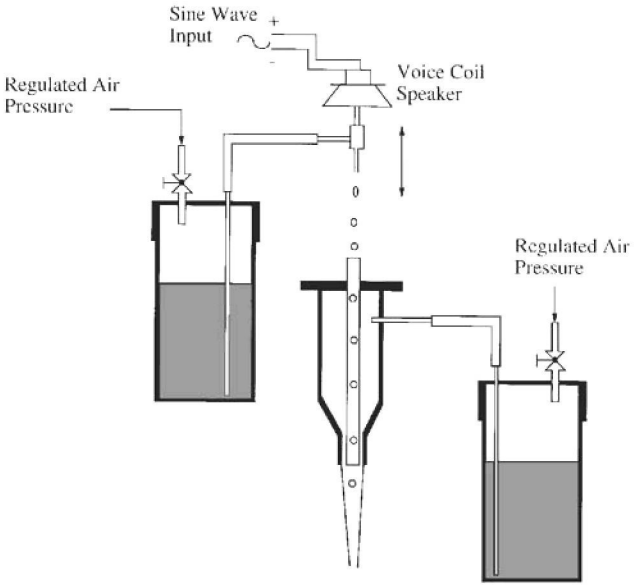

Fig. 4. Schematic of the droplet/falling annulus apparatus. The pressurized vessels on the left and right respectively contain the inner and outer polymer solutions

2.8 Photomicrographs

Optical photomicrographs were taken on an Jena photomicroscope equipped with a B100 M electronic camera (Jena, Germany; supplied by Acts Instruments, Peagram, TN). Illumination was provided by a Cuda Products I-150 illuminator (Acts Instruments). The images were recorded at a shutter speed of 1/125 s on Kodak 400 ASA film. The magnification was 100X.

2.9 pH Measurements

pH was measured with a Sentron 2001 meter (Federal Way, WA).

2.10 Cytotoxicity Testing

RIN 1046–38 rat insulinoma cells were used throughout this investigation [65]. It was believed that a continuous cell line of this type would most closely mimic the β-cells present in pancreatic islets. Cells were cultured in DMEM medium (Cellgro, Mediatech, Herndon, VA) containing 10 vol.% of fetal bovine serum (Hyclone, Logan, UT). Cells were grown in 75-cm^3 tissue culture flasks (Costar, Cambridge, MA) until confluent and then harvested by means of a trypsin/EDTA solution (Gibco/BRL, New York, NY). A 24-well plate (Costar) was employed in all tests. The stock polymer solutions were usually twice the optimal concentration in PBS. 100 µl of cells in DMEM medium were then added to each well. 100, 200 or 500 µl of the polymer solution was pipetted into the well followed by DMEM solution (800, 700 or 400 µl). Each well was homogenized by shaking and the plates were incubated at 37 °C.

Microscopic observations of the cells were carried out daily in order to determine the extent of cell growth and attachment. The color of the growth media was noted as was the presence of any precipitate. A time of 72 h was used as a standard for incubation. The final ranking of cell viability utilized the following scheme. A totally confluent well was given an arbitrary ranking of 7+. Each well was then assigned a percentage confluency which was subsequently converted to a 1+ to 7+ scale. Cells which did not attach to the bottom of the well, or that died, usually floated and were designated with the symbols R (round) or RF (round and floating).

Following the visual inspection, plates were washed with cold PBS (Gibco/BRL) and the cells were treated with 10 mmol/l EDTA/NaOH (pH=11.3) for 20 min at 37 °C [66]. This treatment released DNA. Following removal from the well, the EDTA extract was subjected to a fluorometric DNA assay. A TNE buffer and Hoechst 33258 dye (Polysciences, Warrington, PA) was used to permit DNA determination in the range 10–400 ng/ml. TNE buffer consisted of 2 mol/l NaCl (Fisher, Pittsburgh, PA), 10 mmol/l Tris/HCl (Sigma, St. Louis, MO) and 1 mmol/l EDTA (Sigma). Each measurement was carried out between 3 and 5 times. A TK0100 dedicated mini-fluorometer (Hoefer Scientific Instruments, San Francisco, CA) was used to measure the fluorescence.

3
Results

3.1
Cytotoxicity Screen

From Table 4 it is evident that several natural and modified polyanions did not induce insulinoma cell detachment. Furthermore, the DNA content, a quantitative metric of cell viability, was in general near the baseline (100%) observed in the control studies with PBS. In general both the cell attachment and DNA indices gave similar indications of cytotoxicity. Therefore, in an effort to unify these measures the third column of Table 4 reports an overall ranking of the polymer toxicity toward insulinoma cells on a (-- –, –, +/–, +, ++) scale. The divergent results for the same polymer solution from various suppliers are likely a manifestation of the impurity level with the cytotoxicity of some polymers reduced upon dialysis. The effect of impurities on cytotoxicity will be reported in a subsequent paper [67].

Synthetic polyanions exhibited a moderate level of cell cytotoxicity, with some notable exceptions such as polyacrylic acid and carboxy modified polyacrylamide. The majority of polycations tested seem to be quite non-toxic in contrast to the synthetic quaternary ammoniums which generally exhibited strong cytotoxicity. The only exception to this trend was a polydimethylaminoethylacrylate-*co*-acrylamide, which unfortunately had to be eliminated from further consideration due to the extreme viscosities caused by its high molecular weight ($>10^6$ daltons). It is, however, possible that this polymer could be utilized at lower molecular weights or as a component of a polycation blend. Given these results it appears that the optimal polymer for contact with cell suspensions is a natural polyanion. In the subsequent discussion six natural polyanions will show particular effectiveness in membrane formation (alginate, carrageenan, cellulose sulfate, gellan, hyaluronic acid, xanthan) and these are recommended for further development as the inner constituents of a capsule coacervation system.

As will be demonstrated later in this paper, several of the polycations were suitable for the formation of stable membranes in either binary or quaternary systems, with oligomeric polycations such as polymethylene-*co*-guanidine particularly effective. Their penetration into the polyanionic core was relatively rapid enabling contact of the polycation with the cells prior to the formation of the coacervate complex. Therefore, the cytotoxicity of low molecular weight external polymers should be of concern when selecting polyelectrolytes for encapsulation. Interestingly, there are indications that the polycation toxicity disappears once it is bound in a polymer complex. For example, implants derived from polyelectrolyte complexes based on polystyrene sulfonate and quaternary ammonium polycations [68] have shown no cytotoxicity even though the individual polymers are highly cell toxic. Similar findings have been reported for the haemocompatibility of complex polymers. This implies that the cytotoxicity screen utilized herein overestimates toxicity and a more precise index of cell cytoxicity would be to test each individual polyanion-polycation combination. This would, however, involve adding insoluble complexes to the cell culture media which may reduce

the polymer-cell interactions and invalidate the test. For these reasons, the polyelectrolytes investigated herein were tested individually.

3.2
Effect of Polymer Molecular Weight on Membrane Formation

Table 7 categorizes the type of polyelectrolyte interaction observed between chitosan and polyacrylic acid of various molecular weights. For polyacrylic acid below approximately 20,000 daltons, weak membranes were created. This is likely a consequence of an insufficient number of intermolecular ionic bridges between the chains. As can be expected, as the polyanion size in solution rises a window is observed with respect to the polyacrylic acid molecular weight (20–500 kDa) between which a stable membrane could be produced. As the molecular weight of the acrylic acid was further increased, the higher polyanion solution viscosity reduces the diffusion coefficient of the chitosan into the polyacrylic acid solution. Therefore, the quality of the membrane decreases with increasing polyanion molecular weight until, above one million daltons, the reaction between the polyanion and polycation is so slow that spherical membranes were not produced at all. In addition to the binary chemical interactions between polyions, the molecular weight is an important factor in the type and quality of membrane produced.

3.3
Effect of Polymer Concentration and Solution pH

Given that the polyelectrolytes are being evaluated as potential media for cell suspension and encapsulation, near neutral conditions were imposed on our testing. Therefore, although the dissociation of certain polyelectrolytes such as sodium acrylate is highly pH-dependent, the pH range was limited to between 5.5 and 7.5. Table 8 illustrates the effect of pH and polyion solution viscosity, expressed by the polymer concentration, on the complex coacervation reaction between carboxymethylcellulose and diallydimethylammonium chloride. In Table 8a, one can observe that, at a pH of 7.5, a very small portion of the phase diagram results in stable membranes while a much larger domain corresponds to weak membranes. Clearly the polymer concentration controls both the number of charged groups available for complexation as well as the viscosity of the solution. Therefore, one would expect an optimum at an intermediate concentration. Table 8b illustrates that the stable membrane portion of the domain is significantly enlarged upon reducing the pH to 6.5. This is a consequence of the non-permanent nature of the charge on the carboxymethylcellulose which is favored under acidic conditions. Additionally, Table 8 illustrates that the optimization of the membrane mechanical properties can be contrary to the conditions required for cell viability, which requires near neutral levels of acidity. This is one of many tradeoffs which exist in cell or islet microencapsulation. While lowering the pH did influence the type of polyanion-polycation interaction observed for CMC-pDADMAC, in general, changes in the acidity did not significantly influence the membrane or capsule stability.

Table 7. Characterization of symplexes produced between chitosan and polyacrylic acid of various molecular weights

Polyacrylic Acid Molecular Weight (kDaltons)	Symplex Characteristics
2.1	Weak Membrane
6.0	Weak Membrane
20	Stable Membrane
60	Stable Membrane
140	Stable Membrane
450	Stable Membrane
750	Weak Membrane
1,000	Fibrous Precipitate
4,000	Fine Precipitate

Table 8. Phase diagram illustrating the domains of weak and stable membrane formation

		pDADMAC (pH 7.5)				
	C (%)	2.5	4	10	15	20
	0.1	SOL	SOL	SOL	SOL	SOL
	0.4	PT	WM	WM	SOL	SOL
CMC	0.7	WM	WM	WM	WM	WM
	1.0	PT	WM	WM	WM	**SM**
	1.3	PT	WM	WM	WM	WM
		pDADMAC (pH 6.5)				
	C (%)	2.5	4	10	15	20
	0.1	SOL	SOL	SOL	SOL	SOL
	0.4	WM	WM	WM	SOL	SOL
CMC	0.7	PT	PT	WM	WM	**SM**
	1.0	WM	**SM**	**SM**	**SM**	**SM**
	1.3	PT	PT	WM	**SM**	**SM**

CMC: carboxymethylcellulose; DADMAC: polydiallyldimethylammoinum chloride;
C: concentration of polymer (wt%); SOL: soluble complex;
PT: precipitate; WM: weak membrane; **SM**: stable membrane

3.4 Categorization of Polymer Effectiveness in Membrane Formation

Table 5 summarizes the type of complex produced for each polyanion-polycation pair investigated. There are relatively few systems which yielded soluble complexes (13.6%) with the majority of polyanion-polycation reactions yielding either precipitates (43.7%) or weak membranes (30.7%). Indeed, as one scans across rows of polycations or down columns of polyanions, many of the naturally occurring or synthetic species predominantly form precipitates. This is, perhaps, due to the high content of ionic groups (one per repeat unit) charac-

teristic of polysaccharides. Only 12% of the binary systems tested yielded stable membranes. The percentage of systems which resulted in stable membranes in capsule form was 3.2%. These are indicated in bold in Table 5. While this is a small number (47 systems) it does provide a significant list of alternatives to the classical polylysine-alginate pair which is so common in the literature. In an attempt to quantify a given polymer's effectiveness in membrane formation we have tabulated the fraction of reactions which yielded weak and stable membranes. These results are summarized in Tables 9–12 for naturally occurring polyanions, synthetic polyanions, naturally occurring polycations and synthetic polycations respectively. This index cannot be used to identify an ideal system, although it is useful in delineating the robustness a given polyelectrolyte has as well as in establishing structure-function relationships.

A comparison of Tables 9–12 indicates that naturally occurring species tend to produce stable membranes more abundantly. We believe this is due to the cyclic nature and rigidity of the backbone, as will be discussed in the following section of the paper. Several trends are immediately obvious. For example, the hydrophobicity of the polymer appears to be a critical parameter. While polyacrylic acid can form a stable membrane with virtually all polycations, polymethacrylic acid is much less effective. Similarly, propylene glycol modified alginate is less effective than sodium alginate. Furthermore, when the poly-

Table 9. Percentage of polycations which yielded weak and stable membranes for various naturally occurring polyanions

Polyanion	Percentage of Polycations which yielded a Stable Membrane	Percentage of Polycations which yielded a Weak Membrane
Alginate (Sodium)	8	38
Alginate (Propylene Glycol Modified)	6	34
Carboxymethyl Amylose	0	36
Carboxymethyl Cellulose	17	36
Carboxymethyl Dextran	0	36
Carrageenan (λ)	**42**	**49**
Cellulose Sulfate	**45**	**39**
Chondroitin 4-Sulfate	0	19
Chondroitin 6-Sulfate	3	32
Dextran Sulfate	14	28
Gellan Gum	**65**	**35**
Gelatin A	3	20
Gelatin B	3	40
Gum Arabic	3	12
Heparin	0	34
Hyaluronic Acid	28	58
Polygalacturonic Acid	0	63
Xanthan	**67**	**31**

acrylic acid was copolymerized with the nonionic acrylamide, stable membranes were not produced, presumably because the charge spacing was too large to form a strong polyelectrolyte complex [69]. Other than polyacrylic acid,

Table 10. Percentage of polycations which yielded weak and stable membranes for various synthetic polyanions

Polyanion	Percentage of Polycations which yielded a Stable Membrane	Percentage of Polycations which yielded a Weak Membrane
Pentasodiumtripolyphosphate Hexahydrate	6	8
Polyacrylamide (Carboxy Modified)	0	36
Polyacrylamide (Hydrolyzed)	0	44
Polyacrylamide-*co*-Acrylic Acid	0	25
Polyacrylamide-*co*-Methylpropylsolfonic Acid	17	44
Polyacrylic Acid	**65**	**27**
Polyglutamic Acid	3	17
Polymaleic Acid	3	14
Polymaleic Anhydride	3	25
Polymethacrylic Acid	0	0
Polymethylvinylether Maleic Acid	3	44
Polystyrene Sulfonate	3	31
Polyvinylphosphate	3	9
Polyvinylphonphonic Acid	3	25
Polyvinylsulfonic Acid	3	28

Table 11. Percentage of polyanions which yielded weak and stable membranes for various naturally occurring or modified polycations

Polycation	Percentage of Polyanions which yielded a Stable Membrane	Percentage of Polyanions which yielded a Weak Membrane
Chitosan	**33**	**56**
Chitosan (Glycol Modified)	13	22
Dextran (Diethylaminoethyl Modified)	3	28
Gelatin (Cationic)	8	39
Hydroxymethyl Cellulose (Quaternary)	6	19
Polylysine	8	14

Table 12. Percentage of polyanions which yielded weak and stable membranes for various synthetic polycations

Polycation	Percentage of Polyanions which yielded a Stable Membrane	Percentage of Polyanions which yielded a Weak Membrane
Pentasodiumtripolyphosphate Hexahydrate	6	8
Polyacrylamide (Cationic)	3	56
Polyacrylamide (Cationic) #2	8	67
Polyacrylamide-*co*-Methacryloxy Proplytrimethyl Ammonium Bromide	3	9
Polyallylamine	17	31
Polyamide	6	28
Polyamine	11	44
Polyamine (Quaternized)	17	11
Polybutylacrylate-*co*-Methacryoxyethyl Trimethylammonium Bromide	9	20
Poly-3-chloro-2-hydroxypropyl-methacryloxyethyl dimethylammonium Chloride	14	22
Polydiallyldimethylammonium Chloride	**14**	**44**
Polydiallyldimethylammonium Chloride -*co*-Acrylamide	**14**	**50**
Polydiallyldimethylammonium Chloride-*co*-*N*-Isopropyl Acrylamide	14	6
Polydimethylaminoethyl Acrylate-*co*-Acrylamide (Quaternary)	**17**	**64**
Polydimethylaminoethyl Acrylate-*co*-Acrylamide (Quaternary) #2	14	11
Polydimethylaminoethylmethacrylate (Quat)	11	6
Polydimethylaminoethyl Methacrylate (Quaternized)	9	11
Polydimethylaminoethylmethacrylate-*co*-Acrylamide	6	86
Polyethyleneimine	0	9
Polyethyleneimine-epichlorohydrin	14	31
Polyethyleneimine (hydroxyethylated)	14	17
Polyhydroxymethacryloxyethyl trimethylammonium Chloride	6	19
Polymidazoline (Quaternary)	8	11
Poly-2-methacryoxyethyltrimethyl-ammonium Br	17	11
Polymethyldimethylaminoethylmethacrylate-*co*-Acrylamide	3	69
Polymethylene-*co*-Guanidine	**26**	**11**
Poly-1-methyl-2-vinylpyridinium Bromide	17	19
Poly-1-methyl-4-vinylpyridinium Bromide	17	11
Polyvinylamine	**28**	**64**
Poly-4-vinylbenzyltrimethylammonium Chloride	8	33
Polyvinylpyrrolodone-*co*-Dimethylaminoethyl Methacrylate	3	39

polyvinylsulfone and a copolymer based on methylpropylsulfonic acid were the only other polyanions which produced a significant number of stable membranes. Interestingly, pentasodiumtripolyphosphate hexahydrate and alginate, which form very stable capsules with polyvinylamine and poly-L-lysine respectively were not particularly robust when screened against the entire set of polycations. For naturally occurring polymers trends were also observed. For example, carboxymethyl amylose, an α_{1-4} glucan, was approximately equally effective in the production of membranes as carboxymethyl dextran and dextran sulfate (both based on α_{1-6} glucans). Interestingly, the β_{1-6} glucan (carboxymethyl cellulose and cellulose sulfate) formed membranes more abundantly. Since hydroxide molecules are known to interfere in each other's reactivity of neighboring OH groups [70, 71], the α_{1-4} glucans may be hindered by the isotacticity of their hydroxy group placements. Given this, it is not too surprising that the $_{1-4}\beta$-D glucopyranosyl based xanthan is also very effective in forming membranes with a variety of polycations. Overall eight polyanions can be selected as reasonably robust in their membrane formation capabilities: sodium alginate, cellulose sulfate, hyaluronic acid, xanthan, carrageenans, gellan gum, polyacrylic acid and modified polyacrylic acid. Groboillot et al. have reported a similar list of potential anionic polysaccharides in their review on cell immobilization [72]. With the exception of the latter two synthetic polymers, the majority of the effective polyanions possessed relatively rigid backbones, as indicated by the high value of their Mark-Houwink exponent. This has another advantage since the naturally occurring polyanions tend to be less cytotoxic toward cells (Table 3). Therefore, it would appear that the polyanion would be a more logical choice for the inner polymer in a complex coacervation reaction, as is normally the custom.

There are relatively few naturally occurring polycations available and it became obvious early in our screening that the selection of the appropriate polycation would likely be more limiting than for the polyanion. Indeed, in the literature only chitosan, diethylaminoethyl-chitosan and poly-L-lysine have been evaluated for cell immunoisolation [72]. Tables 11 and 12 show that chitosan, modified chitosan, polyallylamine, polyamine, as well as three quaternary ammonium acrylic polymers were the only macromolecules which performed well across the complete set of polyanions. These represent both flexible and rigid chains with permanent and induced charges. Interestingly, the low molecular weight polymethylene-*co*-guanidine was also effective. The quaternary ammonium diallyldimethyl and dimethylaminoethyl acrylate/methacrylate homo- and copolymers all generated a similar number of membranes. Similarly the polymethylvinylpyridiniums were equally ineffective. Polyallylamine and polyvinylamine, which are chemically very similar to poly-L-lysine, also had similar reactivities with polyanions (Table 5). Polyvinylamine has recently been investigated for cell encapsulation as a pair with alginate [39, 40]. The capsule properties with the synthetic polyvinylamines were also analogous to those obtained for the standard lysine/alginate capsule (i.e. catastrophic rupturing of a fragile membrane with applied stress). Given that polyallylamine and polyvinylamine are available at a fraction of the cost of poly-L-lysine, they seem attractive candidates for further optimization in the way of multicomponent blends.

The poor response of the synthetic polymers in the cytotoxicity tests with insulinoma cells (Table 4) provides further support for the utilization of polyanions as the inner cell suspending fluids. Given the rigid nature of the moderate molecular weight anionic polysaccharides, it seems reasonable that low molecular weight polycations can be effective in membrane formation, due to their high diffusivity. This will be elaborated upon in the discussion.

Table 13 lists the properties of individual polymers for each binary pair which yielded a stable capsule. This, along with Table 4, represent the principal contributions of this paper and their results will be utilized throughout the following discussion.

4 Discussion

4.1 Polymer Attributes to Be Considered in Capsule Formation via Polyelectrolyte Complexation

A preliminary examination of Table 13 reveals that the majority of the 47 binary systems which yielded stable capsules consisted of a polysaccharide inner polymer and a synthetic tertiary amine or quaternary ammonium polycation as the outer material (36 cases). There was only a single example of a synthetic polyanion as a core (polyacrylic acid), while chitosan, a naturally occurring polycation, formed a stable membrane complex with six polyanions, both naturally occurring and synthetic. Polyvinylamine also formed a stable membrane in one instance. Given that chitosan had a predominantly negative biocompatibility with insulinoma cells and cells suspended in polyvinylamine exhibited a limited cell growth response, it appears that the most suitable inner polymer is a naturally occurring polyanion. Indeed, six polysaccharides – alginate, carboxymethyl cellulose, λ-carrageenan, cellulose sulfate, gellan gum and xanthan – were particularly effective in the formation of membranes in combination with various polyamines, polyamides and quaternary ammoniums.

Of the 47 systems which produced a stable membrane, 42 involved an inner polymer which contained a cyclic backbone which was relatively rigid and extended in aqueous solution. For example, xanthan which formed stable membranes with twelve polycations, has a Mark-Houwink-Sakurada exponent of approximately 1.2. These were paired with flexible linear chains. This is typified by systems such as carrageenan/polydimethylamine-*co*-epichlorohydrin, carboxymethyl cellulose/polyallylamine and xanthan/polymethylene-*co*-guanidine. All the suitable inner polymers were found to have molecular weights in the 10^5–10^6 range. However, stable membranes were produced with outer polymers ranging from oligomers to high molecular weight species. We believe that the inner polymer requires a large extended backbone in order to facilitate the interaction with the oppositely charged molecule. Since the membrane is produced as a result of the diffusion of the outer polymer through a spherical inner droplet, the diffusing species should be flexible and have one of the following

Table 13. Summary of all stable microcapsule systems is correlated with the properties of the polymers utilized

Inner Polymer (Listed Alphabetically)	Outer Polymer	Chain Conformation Inner/Outer	Backbone Structure Inner/Outer	Charge Type Inner/Outer	Charge Density Inner/Outer	Molecular Weight Inner/Outer
Naturally occurring polyanions						
Alginate (Sodium)	Polymethylene-*co*-Guanidine	R/F	C/L	I/I	M/M	M/O
Carboxymethyl Cellulose	Polyallylamine	R/F	C/L	I/I	M/H	M/L
Carboxymethyl Cellulose	Polyvinylamine	R/F	C/L	I/I	M/H	M/L
Carboxymethyl Cellulose	Polyamine (Quaternized)	R/F	C/L	I/P	M/H	M/M
Carrageenan (λ)	Polydimethylaminoethyl methacrylate	R/F	C/L	I/P	M/H	M/H
Carrageenan (λ)	Polymethacryloxyethyl triethylammonium Bromide	R/F	C/L	I/P	M/H	M/M
Carrageenan (λ)	Poly(3-chloro-2-hydroxypropyl-methacryloxyethyl-dimethylammonium Chloride)	R/F	C/L	I/P	M/H	M/M
Carrageenan (λ)	Polyamine (Quaternized)	R/F	C/L	I/P	M/H	M/M
Carrageenan (λ)	Polyvinylamine	R/F	C/L	I/P	M/H	M/L
Carrageenan (λ)	Poly-1-methyl-2-vinylpyridinium Bromide	R/F	C/L	I/P	M/H	M/H
Carrageenan (λ)	Poly-1-methyl-4-vinylpyridinium Bromide	R/F	C/L	I/P	M/H	M/H
Carrageenan (λ)	Polydimethylamine-epichlorohydrin Modified (Quaternary)	R/F	C/L	I/P	M/M	M/L
Cellulose Sulfate	Polyallylamine	R/F	C/L	P/I	M/H	M/H
Cellulose Sulfate	Polydimethylaminoethylmethacrylate	R/F	C/L	P/P	M/H	M/H
Cellulose Sulfate	Polyamine (Quaternized)	R/F	C/L	P/P	M/H	M/M
Cellulose Sulfate	Polyethyleneimine (Ethoxylated)	R/F	C/L	P/I	M/M	M/M
Cellulose Sulfate	Poly-1-methyl-2-vinylpyridinium Bromide	R/F	C/L	P/P	M/H	M/H
Cellulose Sulfate	Poly-1-methyl-4-vinylpyridinium Bromide	R/F	C/L	P/P	M/H	M/H
Chondroitin-6 Sulfate	Polyvinylamine	R/F	C/L	P/I	M/H	M/L
Dextran Sulfate	Polyvinylamine	R/F	C/L	P/I	M/H	M/L
Gellan Gum	Poly-L-Lysine	R/F	C/L	I/I	M/H	M/L
Gellan Gum	Polyethyleneimine (hydroxyethylated)	R/F	C/L&B	I/I	M/H	M/L
Gellan Gum	Polyvinylamine	R/F	C/L	I/I	M/H	M/L
Gellan Gum	Polymethacryloxyethyl triethylammonium Bromide	R/F	C/L	I/P	M/H	M/L
Gellan Gum	Polyamine (Quaternized)	R/F	C/L	I/P	M/H	M/H
Gellan Gum	Polyamide (Cationic)	R/F	C/L	I/P	M/M	M/H

Table 13. (continued)

Inner Polymer (Listed Alphabetically)	Outer Polymer	Chain Conformation Inner/Outer	Backbone Structure Inner/Outer	Charge Type Inner/Outer	Charge Density Inner/Outer	Molecular Weight Inner/Outer
Gellan Gum	Polydimethylamino-*co*-epichlorochydrin (Quaternized)	R/F	C/L	I/P	M/M	M/M
Gellan Gum	Polyethyleneimine (ethoxylated)	R/F	C/L&B	I/I	M/M	M/M
Xanthan	Poly-L-Lysine	R/F	C/L	I/I	M/H	H/L
Xanthan	Polyethyleneimine (hydroxyethylated)	R/F	C/L&B	I/I	M/H	H/L
Xanthan	Polyethyleneimine (epichlorohydrin modified)	R/F	C/L&B	I/I	M/M	H/L
Xanthan	Polydiallyldimethylammonium Chloride-*co*-Acrylamide	R/F	C/L	I/P	M/M	H/M
Xanthan	Polyallylamine	R/F	C/L	I/I	M/M	H/L
Xanthan	Polyvinylamine	R/F	C/L	I/I	M/H	H/L
Xanthan	Polydimethylaminoethyl methacrylate	R/F	C/L	I/P	M/H	H/H
Xanthan	Poly(3-chloro-2-hydroxypropyl-methacryoxyethyl-dimethyl ammonium Chloride)	R/F	C/L	I/P	M/H	H/M
Xanthan	Polyamine (Quaternized)	R/F	C/L	I/P	M/H	H/M
Xanthan	Polymethylene-co-Guanidine	R/F	C/L	I/I	M/H	H/O
Xanthan	Poly-1-methyl-2-vinylpyridinium Bromide	R/F	C/L	I/P	M/H	H/H
Xanthan	Poly-1-methyl-4-vinylpyridinium Bromide	R/F	C/L	I/P	M/H	H/H
Synthetic polyanions						
Polyacrylic Acid	Polyethyleneimine (epichlorohydrin modified)	F/F	L/L&B	P/I	H/M	M/L
Polyacrylic Acid	Poly-L-Lysine	F/F	L/L	P/I	H/M	M/L
Naturally occuring polycations						
Chitosan	Alginate (Sodium)	R/R	C/L	I/I	M/M	H/M
Chitosan	Polyglutamic Acid	R/F	C/L	I/I	M/L	H/L
Chitosan	Polystyrene Sulfonate	R/F	C/L	I/I	M/H	H/H
Chitosan	Pentasodiumtripolyphosphate Hexahydrate	R/F	C/L	I/I	M/H	H/O
Synthetic polycation						
Polyvinylamine	Pentasodiumtripolyphosphate Hexahydrate	F/F	L/L	I/I	M/H	L/O
		R: Rigid F: Flexible	C: Cyclic L: Linear B: Branched	I: Induced P: Permanent	L: Low M: Medium H: High	O: Oligo; L: Low M: Medium H: High

attributes – (i) a high diffusion coefficient or (ii) a sufficient number of charged sites for which to form a symplex with the inner polymer. High molecular weight exterior polymers such as the quaternary polyhydroxymethacryloxyethyltriethylammonium bromide have a large number of charged groups and form a dense membrane skin at the outer layer of the capsule, very much akin to asymmetric membranes produced via a phase inversion process. Such observations have also been reported by Groboillot et al. [72]. Conversely, oligomeric species such as the chelant polymethylene-*co*-guanidine have a higher diffusion coefficient which permits the penetration of the inner polymer to a greater depth, although the membrane itself is more porous [73–75]. This deficiency can, however, be corrected in a second, post reactive step which involves a surface coat with a higher molecular weight polymer. It is interesting that stable membranes can be produced with such a diverse range of outer polymers, principally polycations, and a subsequent paper [62] discusses the blending of high and low molecular weight polycations to control simultaneously the mechanical properties and permeability of capsules. In particular, polymethylene-*co*-guanidine was investigated in an attempt to combine the chelating properties of calcium, a typical gelling agent, with the improved long term stability and mechanical properties of macromolecular cations. This finding is akin to Dautzenberg's which shows that capsule mechanical properties can be improved if a broad molecular weight distribution is employed [76]. It remains to be seen if bimodal distributions are superior to unimodal, highly disperse, distributions.

Clearly a strong polyelectrolyte complexation requires a high concentration of ionic groups on both reacting components. Table 13 indicates that all of the 47 stable systems involved an inner polymer that had one charge per repeat unit and an outer polymer which was either moderately (12) or highly (34) charged. This implies that a dense membrane network can be formed if a highly charged extended inner polymer is exposed to an oppositely charged polymer with a charge spacing of approximately the same, or smaller, dimensions. The flexible nature of the outer polymer may permit a conformational adjustment which reduces the effective distance between charges, to form ionic bridges with a larger number of the ions on the inner polymer backbone. Flexible chains, particularly polyelectrolytes of various charge densities, also permit a greater degree of intermolecular interaction. Interestingly, 45 of the 47 inner polymers had charges which were induced by pH while 24 of the outer polymers were permanently charged. This tends to indicate that the membrane formation could be controlled by varying the pH of the solution containing an inner polymer, although in application involving living cells, the suspension should be kept at a near neutral level of acidity. The "normal mode" membranes, prepared with polyanions interior to polycations, generally consisted of a polysaccharide containing a carboxy group whose degree of ionization could be adjusted by increasing the pH. These were paired with either permanently charged quaternary ammoniums (24 systems) or tertiary polyamines with an induced charge (18 systems). By contrast, the "reverse mode" membranes formed with an inner polycation usually possessed a permanent charge on the inner polymer. As a guideline in selecting potential polymer pairs we can report that for only 4 of the 1235 pairs investigated was a stable membrane produced from two perma-

nently charged species. These were all based on cellulose sulfate interior to quaternary ammoniums, a system known to form strong capsular membranes [76]. We can also note that stable membranes were produced for inner polymers where the functional group was attached to the side chain of a cyclic sugar backbone. This indicates that the accessibility of the charge is as important as the concentration and nature of charged species. Ionic groups on the relatively rigid polysaccharide are much more accessible than on a coil. The membrane formation did not appear to correlate with the linearity or degree of branching of the polyion, though a systematic set of polyelectrolyte standards were not available to test this conclusion rigorously. Another important variable in the complexability of oppositely charged polyelectrolytes appears to be the presence of secondary interactions, via hydrogen bonding (Tables 3 and 13). This is not too surprising since both ionic and H-bonding interactions are known to influence the chelation using simple cations.

4.2
Practical Results from the Binary Screening

Several of the capsules which were stable under quiescent conditions, including poly-L-lysine-alginate, ruptured catastrophically under mild deformation. In an effort to improve their mechanical properties, the surface coating of binary polymer capsules was examined. This involved the application of a dilute oppositely charged polyion to saturate the residual surface charges. As most systems contained polyanions interior to polycations, the coating was usually an anionic polyelectrolyte. Table 14 indicates that nine multicomponent capsule systems were identified based on this second level of screening (Steps 5–6 of Table 1). All capsules in Table 14 withstood a compression of 40% without rupturing. Interestingly, beyond this limit the capsule integrity was observed to be strain dependent.

4.3
Thermodynamics of Polymer Complex Formation

The complex coacervation membrane formation process is rather fast (approximately 1 s) and, as such, the resulting capsules are produced under non-equilibrium conditions. It is, therefore, not surprising that post reaction effects such as membrane swelling, shrinking and collapsing occur as the osmotic pressure and activity of the various species equilibrate over the ensuing minutes to hours. If the dialysis effect is significant, the forces involved can compromise the integrity of the membrane by exceeding the ionic interactions. This is particularly true for systems which initially form weak ionic interactions due to a large charge spacing on the polymer backbone. Conversely, for polymers with very strong ionic interactions, a stable complex is rapidly produced in the form of a precipitate and the capsular shape is never attained. Therefore, the optimal membrane formation tends to involve relatively rigid polyanions coupled with flexible polycations. The interacting forces include long range ionic attractions which begin to have an effect for polyions separated by 15 nm and have an energetic minima at

Table 14. List of binary polymer blends which formed stable capsules

Polymer Composition (Inner/Outer/Coating)	Membrane Type	Method of Capsule Formation	Observations
Alginate/ Polyvinylamine/ Alginate	T	A/S A/R	No Fibrotic Growth in Hosts. Permeability 10–100 kDa
Alginate/ Chitosan/ Carboxymethylcellulose	T	D/A	Dissolves in Culture Media
Carboxymethylcellulose (Medium MW/High MW 10:1)/ Chitosan/(No Coating)	T	A/R D/A	–
Carboxymethylcellulose (Medium MW:High MW=10:1)/ Polyallylamine/(No Coating)	T	P/R	–
Carboxymethylcellulose (Medium MW)/Polyvinylamine/ (No Coating)	T	P/R	–
Carrageenan-λ/Polydimethylamine -*co*-Epichlorohydrin (Modified)/ Carboxymethylcellulose	NT	A/S	Permeability 10–75 kDa
Cellulose Sulfate/Chitosan/ (No Coating)	T	D/A	Capsules Fuse Together
Chitosan/ Pentasodiumtripoly-phosphate Hexahydrate/ Carrageenan-λ or Cellulose Sulfate	NT	A/R	Chitosan is Cytotoxic
Chitosan/Polyglutamic Acid/ (No Coating)	T	P/R	Chitosan is Cytotoxic
Gellan/Polyethyleneimine (Hydroxyethyllated)/ (No Coating)	T	A/S	Irregular Shape
Gellan/Polylysine/ (No Coating)in PBS	ST	A/S	Distinct Wall, Float
Polyacrylic Acid/Poly-L-lysine/ (No Coating)	ST	A/S	Irregular Shape
Xanthan/Poly-L-lysine/ (No Coating)	ST	A/R	Distinct Wall, Sticky, Irregular Shape
Xanthan/ Polymethylene-*co*-Guanidine/ (No Coating)	NT	A/S	Very Smooth Surface

A/S: Air Stripping,
D/A: Droplet into a Falling Annulus,
T: Transparent,
NT: Not Transparent.
A/R: Atomization into a Receiving Bath,
P/R: Pipetted Droplets into a Receiving Bath.
ST: Semi-transparent,

approximately 3 nm. Hydrogen bonding also appears to be important since it confers a significant degree of organization to the surrounding water molecules. The organization of the water molecules in a cage around the polymer chains decreases the overall entropy of the surrounding medium. Because such a state is not thermodynamically favorable, shedding (dehydration) of the water is facilitated through additional hydrophobic (van der Waals) interactions between non-charged portions of the polymers. These can be both intra- and inter-molecular. While the electrostatic interactions occur during the initial membrane formation step, dehydration, hydrogen bonding and hydrophobic interactions proceed over much longer time scales, leading to conformational rearrangements and ultimately, conformational stabilization [77].

5 Conclusions

The following guidelines can be utilized for the preparation of stable microcapsular membranes from the complex coacervation of oppositely charged polyelectrolytes.

(1) In general, the optimal inner polymer for a spherical membrane is a highly charged polyanion with a molecular weight in the 10^5–10^6 range with a cyclic backbone and a pH-dependent charge. The charged group is attached as a side chain on the sugar group of a polysaccharide. These properties are obtained with polysaccharides such as alginate, carboxymethyl cellulose, λ-carrageenan, cellulose sulfate, gellan gum and xanthan [78].
(2) Stable spherical membranes (microcapsules) are optimally prepared using flexible polycations of various molecular weights (10^3–10^6 daltons) as the outer polymer. The polycations found most suitable possessed either a permanently charged quaternary ammonium group or a tertiary amine. The formation mechanism, permeability and mechanical properties depend on the molecular weight of the polymers employed, with high molecular weight species requiring a higher content of ionic groups and longer reaction times to compensate for the lower diffusion coefficient. The higher charge density increases coil dimensions, improving the polymer-polymer interpenetration which is reduced at higher molecular weights.
(3) The high diffusion coefficient of low molecular weight polycations renders them particularly effective in membrane formation. This is particularly true for polymeric chelating agents.
(4) Between 50 and 100 mol % of the monomeric units of the outer polymer should contain ionic groups so as to match the spacing of the charges with polysaccharides which have one induceable charge per repeat unit.
(5) The membrane stability appears to be improved due to the presence of secondary interactions, such as those provided through hydrogen bonding.
(6) The formation of stable microcapsules appears to require at least one of the polymer components to have a pH-dependent charge.
(7) Stable microcapsules require the combination of polymer solutions containing both flexible and rigid chains.

(8) Stable membranes are generally produced with polyelectrolytes where the charged group is on a side chain attached to either a cyclic or olefinic backbone.
(9) Synthetic polymers are generally cytotoxic to insulinoma cells.
(10) The solution pH and ionic strength are determined by cell osmotic pressure and viability constraints. Higher polyelectrolytes concentrations generally provide improved membranes although an upper viscosity limit processing creates an optimum dosage for any given polymer.

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Water Soluble Polymers for Immunoisolation II: Evaluation of Multicomponent Microencapsulation Systems

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Microcapsules have been prepared via a complex coacervation reaction from polyanion and polycation mixtures. Multicomponent blends of synthetic, semi-synthetic and naturally occurring macromolecules have been evaluated with a particular interest in the preparation of immunoisolation barriers for pancreatic islets. A screening has resulted in thirty three polymeric systems which have been compared according to their mechanical strength, capsule characteristics (such as shape, surface smoothness, stability, and swelling/shrinking) and permeability (MWCO). A limited number of tests were also carried out on the cell viability in the presence of polymer mixtures. These included measurements of the perifusion of encapsulated pancreatic islets and the host tissue response. The quality of the membrane produced was observed to be a strong function of the polymer properties, processing conditions, such as the type and concentration of the simple electrolyte, and the reaction time. Additionally, a multicomponent polyelectrolyte technology based on the formation of a capsular "wall-complex" was developed for the simultaneous optimization of the membrane mechanical properties and permeability. This involved the preparation of a wide pore matrix through the reaction of a core polyanion with a small ionotropic ion. A second, low or medium molecular weight, polycation was subsequently added, in a second reactive stage, and its time dependent diffusion into the capsule could be used to control the membrane wall thickness and permeability. Alternatively, the simultaneous application of low and high molecular weight cations (or a divalent and polyvalent cation) often led to capsules with similar controllable properties. For many combinations, a distinct capsular wall was observed. Overall seven chemistries were identified as providing suitable permeability and capsular mechanical properties. All contained an anionic polysaccharide blend interior to an oligocation solution. The alginate/cellulose sulfate//polymethylene-*co*-guanidine/calcium chloride/sodium chloride system was found to be the most viable alternative to the standard alginate-polylysine-alginate capsule (APA).

Keywords: Bioartificial pancreas, biomaterials, complex coacervation, immunoisolation, microencapsulation, polyelectrolytes, water soluble polymers

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1 Introduction

1.1 Polymer Blends

In recent years polymeric blends have been recommended as a means of improving polymer properties without markedly changing the structure and function of the dominant polymer, with polymer miscibility understood in terms of the penetration of components on the molecular level. Consequently, a binary blend, or gel, is formed by interpenetrating materials provided the two polymers are not ordered (flexible macromolecules). The backbone flexibility and its role in establishing polymers thermodynamic miscibility has been recognized [1]. Furthermore, blends of rigid and flexible macromolecules are also becoming important. For example a binary gel structure composed of a single polymer network containing the second polymer as a sol within the gel can be produced by

blending a flexible random coil with a macromolecule containing a rigid backbone [2]. The miscibility can be enhanced via hydrogen bonding, or ion-ion interactions. A second, completely non interacting, scenario results from use of molecularly dispersed fillers. Thus, the coil-helix transition (gelation) is markedly accelerated by the addition of a small amount of dextran, methyl cellulose, or polyethylene glycol [3]. At the same time, the acceleration of structure formation by the addition of such polymers is accompanied by the reduction of time required for osmotic equilibration. Indeed, water then functions as a plasticizer [4].

The swelling pressure of a gel is considered to be a balance between osmotic forces which encourage water retention and elastic forces which press the liquid out of the system. Such swelling can be countered by the mechanical forces which maintain, for example, the capsule integrity or by the chemical potential (activity) of the water in a surrounding medium. The chemical potential is influenced by the composition of phosphate buffered saline (PBS) or media used to culture cells. The osmotic swelling for the gel is composed of contributions from all components present in the system, including counter ions and, to a lesser extent, polyelectrolytes (polymers). The mutual exclusion of polymers is an entropic interaction and can contribute to the total osmotic swelling and water retention by the gel [5]. It is particularly important to consider adding a filler (plasticizer) of an inflexible nature so that the proper osmotic balance can be attained.

Coupled networks represent a special case of polymer blends. Agarose (galactan) binding to κ-carrageenan has been observed on the basis of optical rotation and calorimetric data [6]. This is the case of binary gel formation through the interpenetration of two ordered polymers. When the two polymers exist in a blend, the entropy and the internal energy of each component are additive. The conformational change of either of the two polymers in the blend is independent of the other. Thus, the contribution of the polymers to the elastic modulus of the binary gel are additive. Similar observations were made for the co-gelling of agarose and glycans. Specifically, the interaction has been noted between agarose and carboxymethyl cellulose (CMC) or cellulose sulfate (CS) [7].

The information available on aqueous polymer blends is qualitative in nature because of the lack of a suitable theory to interpret the experimental observations. Mixed gels can be comprised of an interpenetrating network, a coupled network (as discussed above), or a phase-separated network [2]. The latter is the most common as the blends have a tendency to form two phases during gelation. In such cases the miscibility and thermodynamic stability have to be empirically investigated and proper conditions for miscible blends identified. This involves a phase diagram study as is described in [3].

The preceding discussion can be used as a guide for rational selection of multicomponent polymer blends used for encapsulation, or as a basis for interpreting our findings.

1.2 Capsular Immobilization Barriers

The majority of the scientific literature describes capsules produced through binary polymer interactions. The most typical capsule is based on alginate-polyly-

sine, with an alginate coating on the exterior, produced using a calcium ion precasting method [9]. Although such capsules have been extensively investigated, they are of limited utility for implants because of their poor mechanical stability. A review of alternative semi-permeable microcapsules prepared from oppositely charged water soluble polyelectrolyte pairs have been compared with 1235 new chemistries in a recent paper [8]. This publication involved structure-property investigations which correlated polymer properties with characteristics associated with the formation and permeability of capsular membranes. The variables investigated included the polymer concentration, molecular weight, pendant groups charge density, the pH of solution, as well as the presence of small ionic species (inorganic salts). The latter is essential for capsule survival in an in vivo application because of the presence of physiological concentrations of salts inside the capsules. The screening program encompassed a combination (matrix) of approximately 36 by 40 polyanions and polycations, respectively [8].

The majority of the aforementioned capsules were either not sufficiently mechanically stable or suffered from other surface or matrix related deficiencies. These deficiencies include poor morphology, such as capsule sphericity and surface smoothness, which result from an osmolar imbalance. Membranes are also often leaky (an internal polymer slowly diffuses out through the capsule wall) or shrink in either PBS or in culture media over a period of a few hours. Exceptionally, some capsules are observed to swell excessively and burst. Furthermore, some complex membranes, although stable in water, dissolve over several days upon a contact with culture media. This is true for pectin based capsules (pectin/calcium salt) and for alginate-chitosan membranes and may be a consequence of the polycation substitution by electrolytes present in the media [10]. In order to improve the existing binary capsules several approaches, both traditional and novel, have been considered and tested herein. These are discussed in the following sections.

1.2.1 *Capsule Coating*

Based on the enhanced stabilization derived from a reduction in the surface charge [11] the mechanical properties can be improved. This method had been employed to seal leaking membranes and the control of membrane permeability.

1.2.2 *Crosslinking*

The crosslinking of polymers in a binary membrane complex *via covalent binding* is a traditional method for improving the impact resistance of materials. Generally, high molecular crosslinkers have been successful (e.g., dextran dialdehyde [12]) while low molecular weight species, such as glutaraldehyde or carbodiimide [1-ethyl-3(3-dimethyl-aminopropyl)-carbodiimide] are considered potentially harmful to cells and are generally avoided [13]. Another fruitful method is to crosslink a membrane *via photopolymerization* as has been successfully applied by several investigators [14, 15].

1.2.3
Chemical Adjustment of Charge Density

The chemical adjustment of charge density is another potentially rewarding technique. It does, however, require either the synthesis of new polymers or the chemical modification of preformed polymers (e.g., supersulfation of hyaluronic acid [16]). This procedure has not been evaluated in our screening.

1.2.4
Combination of Low and High Molecular Weight Polyelectrolytes

Based on our experience, a *combination of low and high molecular weight polyelectrolytes* can be used to adjust the viscosity and reactivity of the reacting mixtures. For example, a combination of lower and high molecular weight carboxymethyl cellulose (CMC) often results in more stable membranes than either polymer individually. Furthermore, the addition of a low molecular species (spermine) to a medium size polylysine leads to sealing of leaky membrane as well as to adjustment of membrane permeability. Indeed, the penetration of the highly reactive small cation through preformed polylysine-alginate complex is limited and forms a distinct membrane on the top of capsules when added sequentially. Similarly, a high molecular species reduces the penetration of a low molecular mass species within the capsule, minimizing a possible inhibitory effect of the usually highly cytotoxic oligomeric cations.

1.2.5
Adjustment of Osmotic Pressure

The replacement of PBS, or a 0.9% saline solution, by mannitol or sorbitol, of the same osmolarity as is the physiological solution, in order to minimize the charge screening of the polymer complex represents another possible direction to increase the strength of the complex. Matthew et al. [17] have applied a mannitol solution for the encapsulation of hepatocytes, showing that the mixture of CMC and chondroitin sulfate C formed a stable complex with chitosan in the absence of salts.

1.2.6
Polymer Grafting

Polymer grafting can be used to alter chemical and physical properties of a homopolymer. For example, Sawhney and Hubbell [18] grafted polyethyleneoxide to poly L-lysine to enhance biocompatibility of polylysine and improve the polylysine-alginate capsules. Stevenson and Sefton [19] modified alginate by grafting it with hydroxyalkyl methacrylate, again to improve the biocompatibility and to allow for polymerization by means of γ-irradiation. Covalently modified (*co*)-polymers have not been evaluated in this study.

1.2.7 *Polymer Blending*

Polymer blending (composites, mixed gels) has been considered for both internal (core) and external polymer solutions. Both ionogenic and nonionic polymers have been investigated as components of blends. For example, non charged dextran has been added to a simple electrolyte solution (calcium chloride) to facilitate the formation of perfectly spherical alginate beads [10]. Such a polymer is often termed a "viscosity enhancer" or "filler". Others [20] have used a combination of alginate with hydrophobically modified alginate (PGA, propylene-glycol alginate) as the core polymeric material to increase the capsule permeability, in a reaction with chitosan and calcium salts in a receiving bath. The PGA interferes with the alginate-chitosan electrostatic interaction process and allows for higher membrane porosity. At the same time, nonionic polymer can also be used to adjust capsule osmotic environment and permeability. Water retention by rather inflexible and fully extended polysaccharides is due to a large excluded volume effect. Such an effect can be used to adjust osmotic pressure within a capsule subjected to shrinking. For example, Philipp et al. [21] used a mixture of CMC (filler) and cellulose sulfate on the anionic side with polyethylene imine to control the water flux (and osmotic pressure) and permeability of cast membranes. None of the capsules mentioned above were sufficiently stable.

A special case of polymer blending is represented by a combination of thermosetting and ionotropic core polymers. Among thermosetting polymers, low-temperature gelling type agarose, a largely uncharged polymer, can be listed. Examples of ionotropic polymers include alginate and κ- and ι-carrageenans (potassium or calcium salts). The resulting mixed gels are very strong and often flexible. One potential disadvantage of polymer blending is the possible incompatibilization of polymer solutions.

1.2.8 *Bead Processing*

Bead precasting, also known as the template method [10] or polymerization mold method [22], involves the development of a membrane on the exterior of the bead. It is considered the most promising approach, and involves a multi-step process. First a stable bead is generated from an ionotropic polysaccharide solution. In order to provide an osmotically friendly environment for cells, physiological salt solution or PBS can also be employed to dissolve gelled alginate. Mannitol or sorbitol serve a similar role for the carrageenans (κ- and ι-). A second reactive step involves the generation of a permeable "wall" which will be subsequently discussed. The main reasons for precasting are as follows: (1) a stable membrane can be produced on the top of a pre-formed bead, composed of polyelectrolyte pairs which form poor symplexes, and (2) the gelled bead, or a gel layer on the top of the capsule, limits the permeability of the polymeric cation inside the capsule and thus minimizes its potential harmful effect on cells.

The bead precasting is then followed by a wall build-up step which involves the addition of a complexing polyelectrolyte. Typically, a visible wall is generated

(10–100 nm), though the barrier properties are specific to the polymer chemistry. The wall thickness can be controlled by varying the concentration of polycation used or by changing the reaction time. The gelled bead core can then be liquefied if a convenient chelating agent is available. For example, EDTA can be used to remove divalent ions. For other capsules a gelled interior can be retained provided it is acceptable to the particular cell line. This approach has been the most rewarding in the present study and was used in combination with other improvements (Sects. 1.2.4, 1.2.5 and 1.2.7). While some "walled" capsules have been presented in literature, our novel approach, emphasizing bead precasting with a wall build-up, offers very stable and biocompatible membrane systems. This permits a high degree of permeability control as well as the decoupling of permeability and mechanical properties, a limitation of the binary polyelectrolyte systems.

2 Experimental

2.1 Polymers

All polymers utilized in this investigation have been listed in part I of this publication series, as have the methods of solution preparation [8].

2.2 Preparation of Capsules/Beads

A complete description of droplet generator and of several atomization methods appears in a previous paper [8]. Simple air-stripping or piezoelectric drop generators were employed. The core liquid typically consisted of a polyanion solution, while the receiving bath contained a polycation(s) solution and, in many instances, a divalent cation.

2.3 Permeability Measurement: Efflux Method

Capsules were equilibrated with a tracer solution overnight. A capsule pellet (0.2–0.5 ml) was then placed in 5 ml test buffer (PBS or RPMI-1640 medium, Gibco/BRL, New York, NY) on a shaker and a 0.2-ml aliquot was immediately sampled by a screen-protected pipette with further samples being taken over the next 700 s. The tracer quantity was assayed using the methods described below. A final sample was taken after the capsules has been in contact with the buffer for several hours (equilibrated tracer quantity) and the increment to the tracer concentration at each time was calculated. From the progress of tracer to equilibrium on a semilog plot a slope denoted as the zero-order rate flux constant was obtained and has been used as a measure of capsule permeability. [^{3}H]-Glucose (580 daltons), insulin (6.2 kDa), and ovalbumin (45 kDa) have been used as tracers. Radioactivity was measured by means of a Packard 2000CA Liquid Scintillation Counter (Packard Instruments,

Dowers Grove, IL), insulin by a radio immunoassay by means of Coat-A-Count Insulin Detection Kit (Diagnostic Products Corp., Los Angeles, CA), and proteins by Bio-Rad Protein Assay (Bradford) method (Bio-Rad, Hercules, CA).

2.4 Permeability Measurement: Inverse Gel Permeation Chromatography (IGPC)

In addition to the efflux method, several experiments have been performed by means of an inverse GPC method to assess the capsule permeability. Such measurements are considered to be more accurate and representative for high molecular weight species since they are not sensitive to protein adsorption on the capsule surface. This method, however, cannot be used for a massive screening of capsule permeability due to its time requirements.

The membrane permeability and mass transfer characteristics of capsules were measured by packing approximately 10 ml of capsules into a sterile polyethylene syringe. This packed bed was attached to a Hitachi L6000 isocratic HPLC pump (Hitachi Instruments, Tokyo, Japan) at a flowrate of 0.1 ml/min. The mobile phase was an aqueous solution containing a buffer (PBS). Standards of polyethylene oxide were injected into the mobile phase via a Rheodyne 7725I injector (Coati, CA) and the polymer concentration was monitored via a Hitachi 4000H UV detector operating at 214 nm. To avoid damage to the capsules, the flow was counter-gravity. Figure 1 presents an example of a permeability measurement using the inverse GPC method.

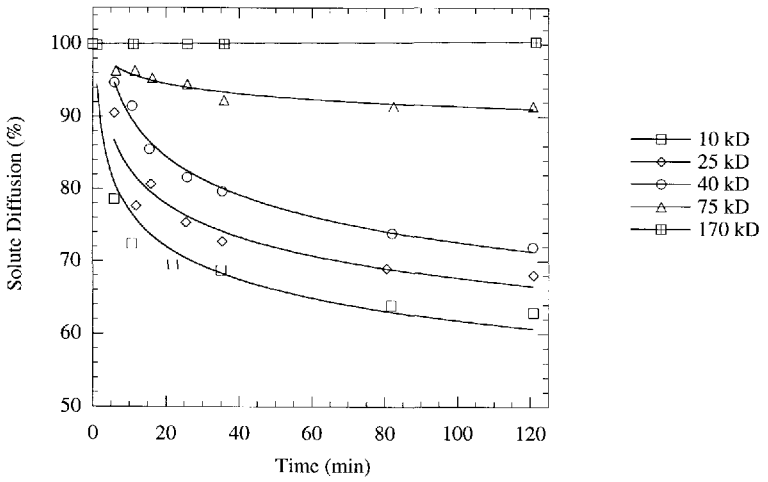

Fig. 1. Capsule permeability as measured by the inverse GPC method. Capsules were made from 1.25% λ-carrageenan (Fluka) and 0.02% carboxymethylcellulose (Aqualon) in 0.9% sodium chloride (core polymers) and 2% polydimethylamine-*co*-epichlorohydrin modified, quaternized (Scientific Polymer Products) and a quaternary amine (Agefloc B50, CPS) in PBS (receiving bath) using a 3 min reaction time. The capsules were subsequently washed with PBS, coated for 15 min with 0.1% LV alginate (Kelco) and again washed in PBS. Two molecular size dextrans were used to probe the capsule permeability. 170 kD dextran is almost totally excluded while the lower molar mass polymers permeated the membrane to varying extents

2.5
Islet Isolation and Perifusion

Pancreatic islets were isolated from male Sprague-Dawley rats (250–285 g, Harlan). The pancreatic duct was inflated with a solution of Hank's Balanced Buffer Solution (HBBS; Gibco/BRL, New York, NY) containing collagenase (Boehringer-Mannheim, Indianapolis, IN; Collagenase P). Groups of three pancreases were digested in 2 mg/rat collagenase in HBBS for 6–13 min at 37 °C using a wrist-action shaker. The digestion was stopped by the addition of cold HBBS with 10% Newborn Calf Serum (NCS; Hyclone, Logan, UT) and shaken vigorously for 10–15 s. The digested material was washed three times with cold HBBS and filtered through a wire mesh cell strainer to remove undigested material. Pancreatic islets were separated using a 11%–20.5%–23% Ficoll (Sigma) gradient and stored in University of Wisconsin Storage solution for 19–24 h prior to encapsulation.

Insulin secretion by encapsulated rat islets was evaluated in a perifusion apparatus with a flow rate of 0.1 ml/min with RPMI-1640 with 0.1% BSA (Sigma) as a perifusate. Encapsulated islets were perifused with 2 mmol/l glucose for 30 min and the column flowthrough discarded. Three minutes samples of perifusate were collected during a 30 min perifusion of 2 mmol/l glucose, a 30 min perifusion of 20 mmol/l glucose+250 mM IBMX, and a 60 min perifusion of 2 mmol/l glucose. Samples were assayed in duplicate for insulin using Coat-A-Count Detection Kit with a rate insulin standard.

2.6
Transplantation of Capsules and Beads

Empty capsules or capsules containing rate islets, in a packed capsule volume of 0.2–0.5 ml, were sterilely transplanted into the peritoneal cavity of metofane-anesthetized mice. The mice were allowed to recover and maintained for 1–3 months. At certain times following transplantation, mice were sacrificed and capsules were retrieved through a peritoneal lavage.

3
Results

3.1
Experimental Design

In a previous publication [8] we described a systematic screening of the binary interactions between 36 polyanions and 40 polycations. As a result of this study it became clear that capsules prepared from simple binary polymer complexes would not be mechanically adequate and multicomponent polymer systems would offer advantages. The rationale for capsule improvement, and for the use of a multicomponent system, has been presented in the Introduction. We have elected to investigate the methods outlined in Sects. 1.2.7 and 1.2.8 (polymer

blending and bead precasting) as the most perspective techniques, together with those discussed in Sects. 1.2.4 and 1.2.5 (a combination of low/high molecular weight polymers and the adjustment of osmotic pressure by means of nonmetabolizable sugars). The use of mannitol or sorbitol was necessary to prevent ionotropic gelling of some polyanions used to precast beads. As a starting point for the investigation the most optimal 47 binary systems were selected [8]. In addition, several polymers have been added to the multicomponent screening which had not previously been systematically studied. These primarily involved small molecular cationic species such as spermine, protamine sulfate, salmine sulfate, lysozyme, polybrene, as well as carrageenan (κ- and ι-), collagen and low temperature melting agarose (nonionic). Clearly, any systematic testing of multicomponent systems would be impossible. To simplify our search, the majority of the multicomponent systems involved:

1) a precast of bead with the subsequent formation of membrane surface;
2) the simultaneous use of small divalent cations with high molecular weight polycations; or
3) a blend of low and high molecular weight polycations.

Typically, a binary system was selected as the base component of the recipe and the addition of polyelectrolytes to either side (core or receiving bath) was tested to evaluate the change in the capsule properties. The 33 successful multicomponent membrane systems are presented in Table 1. The components of the core material side (21 different chemical compositions) are listed in the first column, while the receiving bath components (20 different chemical compositions) are listed in the second column. With the exception of xanthan and CMC, the first polymer listed on the core side are gelling polymers which form beads with the appropriate ionotropic cation (salt). CMC can also be gelled by ions (alum), although they are considered to be non-compatible for cellular applications. The cations were tested both sequentially, usually with ionotropic cation first, and simultaneously. Walled capsules with adequate mechanical properties were often obtained through the simultaneous application of two polycations. Such a

Table 1. Successful multicomponent membrane systems

Polyanion Blend No.	Composition of Polyanion Blend	Composition of Polycation Blend	Polycation Blend No.
1	HV Alginate/Cellulose Sulfate	Polymethylene-*co*-guanidine/ Calcium Chloride	1
1	HV Alginate/Cellulose Sulfate	Polydimethylamine-*co*-epichlorohydrin modified,quaternary	2
1	HV Alginate/Cellulose Sulfate	Polyvinylamine/Calcium Chloride	3
1	HV Alginate/Cellulose Sulfate	Polylysine/Calcium Chloride	4
1	HV Alginate/Cellulose Sulfate	Polyvinylamine(LMW & HMW)	5

Table 1. (continued)

Polyanion Blend No.	Composition of Polyanion Blend	Composition of Polycation Blend	Polycation Blend No.
2	Alginate/Cellulose Sulfate/ Collagen	Polymethylene-*co*-guanidine/ Calcium Chloride	1
3	Alginate/Carrageenan κ/LTM-Agarose	Protamine sulfate/Calcium and Potassium Chloride	6
4	Alginate/Carrageenan κ and λ	Protamine sulfate/Calcium and Potassium Chloride	6
5	Alginate/Carrageenan κ	Protamine sulfate/Calcium and Potassium Chloride	6
6	Alginate/Cellulose Sulfate	Spermine/Polydimethylene-*co*-guanidine	7
7	Alginate	Spermine/Polydimethylene-*co*-guanidine	7
8	Alginate/LTM Agarose	Polybrene/Calcium Chloride	8
9	HV Alginate/Gellan	Protamine sulfate/Calcium and Potassium Chloride	6
9	HV Alginate/Gellan	Lysozyme/Calcium Chloride	9
10	Carrageenan κ and ι	Protamine sulfate/Calcium and Potassium Chloride	6
11	Carrageenan κ/Hyaluronic Acid	Protamine sulfate/Calcium and Potassium Chloride	6
11	Carrageenan κ/Hyaluronic Acid	Polybrene	10
12	Carrageenan κ/Chondroitin Sulfate A	Polyvinylamine(LMW & HMW)	5
13	LE-Pectin/Cellulose Sulfate	Polymethylene-*co*-guanidine/ Calcium Chloride	1
13	LE-Pectin/Cellulose Sulfate	Quart-Polyamine/Calcium Chloride	11
14	LE-Pectin	Quart-Polyamine/Calcium Chloride	11
15	LE-Pectin/LTM-Agarose	Polybrene/Calcium Chloride	8
16	Xanthan	Polylysine/Spermine	12
16	Xanthan	Polylysine/Polyallylamine	13
16	Xanthan	Polyvinylamine/Polyethyleneimine hydroxyethylated	14
17	Xanthan/Alginate	Polymethylene-*co*-guanidine	15
18	Xanthan/Cellulose Sulfate	Polymethylene-*co*-guanidine	15
19	Carboxymethylcellulose (HMW)/Cellulose Sulfate	Chitosan(LMW)/Polyvinylamine	16
20	CMC(HMW)/Hyaluronic Acid	Chitosan(LMW)/Polyethyleneimine-hydroxylethylated	17
20	CMC(HMW)/Hyaluronic Acid	Chitosan(LMW)/pDADMAC	18
20	CMC(HMW)/Hyaluronic Acid	Chitosan(LMW)/Quaternary-Polyamine	19
20	CMC(HMW)/Hyaluronic Acid	Chitosan(LMW)/ Polydimethylamine-*co*-epichlorohydrin modified quaternary	20
21	CMC(HMW)/Carrageenan λ	Polymethylene-*co*-guanidine	15

LE: Low Esterified
MMW: Medium Molecular Weight
HMW: High Molecular Weight
LTM: Low Temperature Melting
LMW: Low Molecular Weight
HV: High Viscosity

Table 2. Properties of selected multicomponent membrane systems

Polyanion Blend	Polycation Blend	Membrane Type	Comment	Blend Nos.* from Table 1
0.15% Xanthan	0.025 Poly-L-Lysine (55 kd)/ 2% Spermine Sulfate	ST	Irregular Shape, Discreet Wall, Float in PBS, Poor Mechanical Stability	16/12
0.15% Xanthan	0.025% Polylysine/ 5% Polyallylamine	NT	Spherical, Smooth, Good Mechanical Stability	16/13
0.12% Keltrol Xanthan/0.3% Cellulose Sulfate	10% Polymethylene-*co*-guanidine	NT	Smooth, Good Mechanical Stability	18/15
0.8% HV Alginate/ 1% Cellulose Sulfate	3% Polymethylene-*co*-guanidine/ 1% Calcium Chloride	NT	Smooth and Swollen, Distinct Wall	1/1
0.8% HV Alginate/ 0.5% Cellulose Sulfate	4% Polydimethylamine-*co*-epichlorohydrin modified quaternary	NT	Spherical, Smooth	1/2
1.6% LV Alginate/ 0.5% Cellulose Sulfate	1.3% Spermine/6.5% Polymethylene-*co*-guanidine	NT	Irregular Shape	6/7
1% HV Alginate	2% Spermine/1% Polymethylene-*co*-guanidine	T	Smooth, Mosaic Membrane (Polymer Incompatibility?)	7/7
1% HV Alginate	1% Spermine/5% Polymethylene-*co*-guanidine	NT	Smooth	7/7

T: Transparent ST: Semi-Transparent NT: Non-Transparent

method is also simpler, since it involves fewer processing steps. Table 2 lists some specific conditions for capsule/beads formation as well as the capsule and membrane properties.

3.2 Permeability Screen

Tables 3 and 4 present a summary of the permeability screening data. In both tables the first entry is considered to be our "standard" capsule and its permeability is used to scale the remaining entries. The first capsule in Table 3 (alginate/cellulose sulfate//polymethylene-*co*-guanidine/calcium chloride) is quite permeable and exhibits good performance in islet encapsulation, perifusion, and implantation studies (see discussion below). By comparison the "standard" capsule in Table 4 (with the same chemistry as above) has a tighter membrane due to the 50% increase in the reaction time, as is revealed from small values of rate constants. In our opinion, the permeability is more appropriately judged through the diffusion of high molecular weight species, rather than through glucose or insulin, since these are the molecules the immunoisolation barrier must block.

Table 3. Permeability data for selected capsules

	System		Reaction Time	Zero-Order Rate Constant (min^{-1})		Overall Ranking
	Anion Blend	Cation Blend	(min)	Insulin (6.2 kdaltons)	Ovalbumin (45 kdaltons)	
1	Alginate(HV,0.6%)/ Cellulose Sulfate(0.6%)	Polymethylene-*co*-guanidine(1%)/ $CaCl_2$(1%)	1	0.29 (100%)$^+$	0.18 (100%)$^+$	W,*
2	Alginate(HV,0.6%)/ Cellulose Sulfate(0.6%)	Polymethylene-*co*-guanidine(2%)/ $CaCl_2$(1%)	0.5	0.07 (26%)	0.009 (5%)	W
3	Gellan(0.6%)/HMP(0.2%)/ Alginate(HV,0.25%)/mannitol(3.6%)	$CaCl_2$(1%)	10	0.507 (175%)	0.10 (55%)	*
4	Gellan(0.6%)/HMP(0.2%)/ Alginate(HV,0.25%)/mannitol(3.6%)	Lysozyme(1%)/$CaCl_2$(1%)	5	0.557 (198%)	0.11 (61%)	*
5	LE-Pectin(4%)	Polyamine(Quaternerized,1%)/$CaCl_2$(1%)	5	1.241 (428%)	0.39 (217%)	
6	LE-Pectin(3%)/Agarose IX-A(1%)	Polybrene(1%)/$CaCl_2$(2%)	5	0.41 (141%)	0.21 (117%)	*
7	κ-Carrageenan X52(1%)/ mannitol(3.6%)	Protamine Sulfate(0.2%)/$CaCl_2$(1%)	5	0.32 (110%)	0.001 (0.5%)	*
8	κ-Carrageenan X52(1%)/ Hyaluronic Acid(0.1%)/mannitol(3.6%)	Protamine Sulfate(0.5%)/$CaCl_2$(1%)	2	0.0276 (95%)	0.006 (3.3%)	W
9	κ-Carrageenan X52(1%)/ Hyaluronic Acid(0.1%)/mannitol(3.6%)	Polybrene(1%)	5	0.369 (127%)	0.13 (72%)	W,*
10	κ-Carrageenan X52(1%)/ Hyaluronic Acid(0.05%)/mannitol(3.6%)	Protamine Sulfate(0.5%)/$CaCl_2$(0.5%)	2	0.36 (124%)	0.01 (5.5%)	W

HMP: Hexamonophosphate W: Wall Visible *: Good Candidate $^+$: Set at 100% for the 'Standard' Capsule

Table 4. Permeability data for selected capsules with glucose as a permeate

	System		Reaction Time	Zero-Order Rate Constant (min^{-1})			Overall Ranking
	Anion Blend	Cation Blend	(min)	Glucose (580 daltons)	Insulin (6.2 kdaltons)	Ovalbumin (45 kdaltons)	
1	Alginate(HV,0.6%)/ Cellulose Sulfate(0.6%)	Polymethylene-*co*-guanidine (1%)/$CaCl_2$(1%)	1.5	0.32	0.049 (100%)$^+$	0.031 (100%)$^+$	W,*
2	κ-Carrageenan(0.5%)/ Alginate(HV,1%) Sorbitol(3.6%)	Protamine Sulfate(1%)/ $CaCl_2$(0.5%)/KCl(0.5%)	5	0.30	0.05 (10%)	0.001 (3%)	W
3	κ-Carrageenan(0.5%)/ Hyaluronic Acid(0.25%) Sorbitol(3.6%)	Protamine Sulfate(1%)/ $CaCl_2$(0.5%)/KCl(0.5%)	5	0.30	0.026 (53%)	0.028 (90%)	W
4	κ-Carrageenan(0.5%)/ λ-Carrageenan(0.5%)/ Alginate(HV,1%) Sorbitol(3.6%)	Protamine Sulfate(1%)/ $CaCl_2$(0.5%)/KCl(0.5%)	5	0.42	0.0066 (13%)	0.001 (3%)	W
5	Gellan(0.6%)/ HMP(0.2%)/ Alginate(HV,0.5%)/ Sorbitol(3.6%)	Protamine Sulfate(1%)/ $CaCl_2$(0.5%)/KCl(0.5%)	3	0.42	0.031 (63%)	0.011 (35%)	W, *

HMP: Hexamonophosphate W: Wall Visible *: Good Candidate $^+$: Set at 100% for the 'Standard' Capsule

In Table 3, the membranes of capsules #2, 7, 8, and 10 are quite dense and have low permeability. In Table 4, capsule entries #2 and 4 are again relatively impermeable and are probably unsuitable for xenogeneic cell encapsulation. By comparison the "alginate/cellulose sulfate//polydimethylene-*co*-guanidine/calcium chloride" capsules seem to offer the most suitable MWCO (approximately 100 kD). This type of capsule is photographed in Fig. 2, with

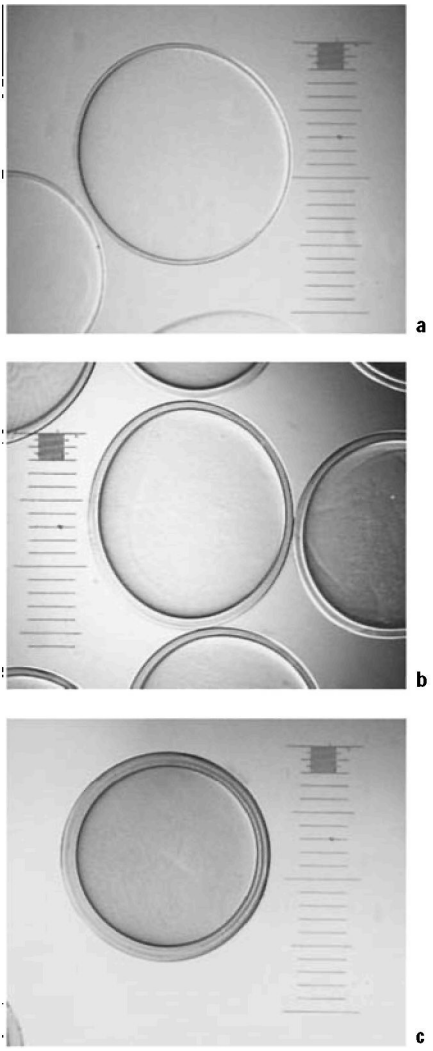

Fig. 2a–c. Morphology of an empty capsule. Capsules were produced from 0.6% HV alginate/0.6% cellulose sulfate/1.2% polymethylene-*co*-guanidine/1% calcium chloride. The reaction time was 30 s. The capsule size is approximately 1.60 mm while the membrane thickness is 0.033 mm; **a** the wall complex is clearly evident; **b** progression in the membrane thickness 0.073 mm with reaction time 180 s; **c** progression in the membrane thickness 0.106 mm with reaction time 300 s

the dependence of the membrane thickness on reaction time evident. The permeability, in general, depends on the concentration of components and reaction time, with longer reaction times resulting in more extensive ionotropic gelation. The wall build-up, for capsules exhibiting microscopically visible wall, also increases with the contact time between the oppositely charged electrolytes. Therefore, the wall thickness can easily be controlled for a given multicomponent blend and varied from a fragile thin shell to a completely penetrated bead. Thus an inherent advantage of the precasting method is that it permits the decoupling of mechanical strength and permeability. In binary symplexes an increase in mechanical properties usually results in a lowering of the MWCO. The control of the permeability in a second step is an important advantage of the precast bead technology. The capsules/beads with reasonably thick walls (30–100 mm) are considered by the authors to be the most suitable for applications.

In addition to gelation induced by divalent ions, thermosetting gelation with low temperature melting (LTM) agarose offers certain advantages. The use of LTM agarose was found to be necessary to maintain a relatively nongelling polymer mixture slightly above room temperature. The extent of the ionotropic gelling was controlled by the relative and absolute concentrations of simple ions and the ionotropic gelling polymer. The effect of reaction time was found to be important provided it was in the 10–30 min range. In most cases, simple ions were left inside the beads as only calcium could be removed by a chelating agent (EDTA). It was postulated that the appropriate combination and concentration of ions could be subsequently optimized to minimize any potential cell damage. An asterisk in Tables 3 and 4 denotes a multicomponent quaternary polymer system which has been recommended for further study.

3.3
Perifusion and Implantation Studies

Figure 3 presents an example of islet functioning inside a particular capsule. A typical two-phase perifusion profile is noted, similar in quantitative terms to that of unencapsulated islets. Clearly the ratio of the membrane thickness to capsule diameter is an important parameter, with low membrane:capsule ratios providing rapid transfer of nutrients and the exodiffusion of insulin. Contrarily, for thick walled capsules of diameter less then approximately one-half millimeter the perifusion response, as measured by the stimulation index and retardation in insulin response to a glucose stimulus, is slower for encapsulated islets relative to free islets.

With regard to capsule transplantation, no ill effect on animal growth or behavior was noted as a result of capsule transplantation. Some capsules were free-floating within the peritoneal cavity and were free of fibrosis on the surface while others were adherent to the peritoneal membrane or to each other. The latter criteria served for further selection and screening of capsule chemistries. Details on the in vivo functioning of encapsulated islets have been presented elsewhere [23].

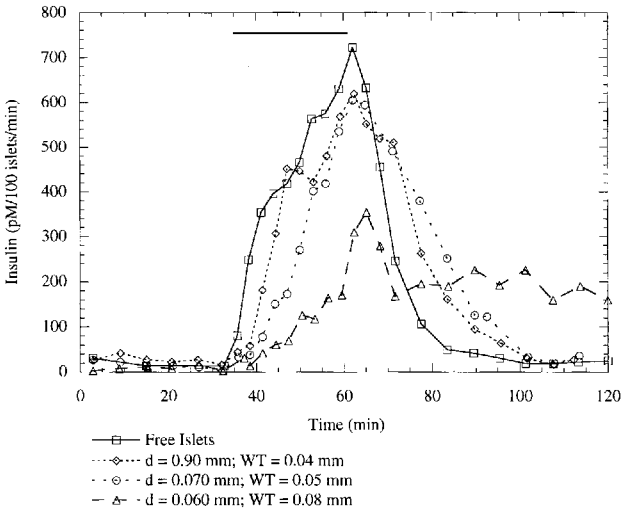

Fig. 3. Secretion of insulin by encapsulated rat islets. Islets were evaluated in the perifusion system following stimulation with 20 mmol/l glucose and 0.25 mmol/l IBMX (note a bar on the X-axis). Insulin was measured by radioimmunoassay. Alginate/cellulose sulfate capsules were made from 0.6% HV sodium alginate (Kelco) and 0.6% cellulose sulfate (Janssen) in PBS (core polymers) and 1% polydimethylene-*co*-guanidine hydrochloride (Scientific Polymer Products) and 1% calcium chloride in 0.9% sodium chloride (receiving bath), using a 1.5 min reaction time. They were then washed with PBS, coated with 0.1% LV alginate (Kelco) for 15 min and again washed with PBS. The response of the alginate/cellulose sulfate capsules was almost identical to that of free islets (control), although the stimulation index and delay in the onset of insulin exodiffusion decreases as the ratio of the membrane thickness to capsule diameter rises

4 Discussion

4.1 Evaluation of Various Multicomponent Systems

In general, seven polysaccharides (alginate, carboxymethyl cellulose, carrageenans, cellulose sulfate, gellan, pectin, and xanthan) were found to be the most effective polymers as the "inner" material as shown in Table 1. As has been discussed in a previous publication [1], the core material is preferably a moderate-to-high molar mass rigid polyanion with one permanent charge per repeat unit. Flexible chains, such as synthetic polymers, were not found to be suitable in any instances. The functional group attachment to the inner polymer is preferably via a side chain and hydrogen bonding seems to be required as a second force to complement the ion-ion binding. The blending of two polyanions to form the droplet core is primarily required for rheological considerations. As a typical example, blends of alginate and cellulose sulfate or carboxymethylcellulose/carrageenan with hyaluronic acid were more effective in yielding mechanically stable capsules then any of these polysaccharides individually (Table 1).

Two categories of polycations were found to be effective as the constituents of the "outer" polyelectrolyte solution. Oligomeric species, in combination with divalent cations, were particularly useful in the decoupling of mechanical properties from permeability control. As is exemplified by the first entry in Table 1, an alginate-cellulose sulfate blend was gelled in the presence of calcium chloride. In a second reactive step the oligomeric polymethylene-*co*-guanidine was added. Its diffusion into the pre-cast capsule could be temporally regulated with longer reaction times reducing the membrane MWCO. Several similar capsules were also produced using low molecular weight amines such as spermine and protamine sulfate as well as polybrene (Table 1). It is quite possible that an oligomeric cationic species forms a special type of complex with two polyanions. It has been shown recently that three-component interpolymer complexes can be formed between two polyanions and low molar mass cation (polyacrylic acid and sodium polyphosphate on one side and a dibasic vinylpyridine on the other) [24].

Moderate molar mass (10^{4-5} daltons) polycations such as lysine and polyvinylamine were also effective in producing capsular membranes. In this case a thin symplex wall is produced as a result of the matching of the charge separations on the oppositely charged polyelectrolytes. In general the outer polymer required a flexible material, generally with a permanent charge. Rigid or high molar mass polymers were unable to rearrange on the time scale required to produce suitably strong membranes.

Table 2 indicates that the most suitable capsular membranes comprised semi- or non-transparent systems. Generally, the multicomponent blending resulted in smooth capsules with the exception of the alginate/spermine-polymethylene-*co*-guanidine systems which were either irregularly shaped or mosaic. There was no correlation observed between the capsule turbidity and permeability.

If we take the alginate/cellulose sulfate//polymethylene-*co*-guanidine/calcium chloride as a base case which provided good permeability to insulin and ovalbumin (Table 3), then it is clear that several alternative capsules can be prepared with similar mass transfer characteristics. Therefore, the newly proposed precasting technology, coupled with multicomponent polysaccharide blends, does permit the control of permeability and diffusion. We believe this to be an advantage. A comparison of the first rows in Tables 3 and 4 indicates how the precise control of reaction time can be used to adjust the permeability. Moreover, rows 2 and 4 in Table 4, both involving κ-carrageenan and 5 min reaction time, are much less permeable than alginate or gellan based systems reacted for shorter durations. Therefore, by employing multicomponent blends and controlling the reaction time, mechanically stable permeselective alternatives to the standard alginate-polylysine-alginate capsules (APA) can be produced from several polymer chemistries. In particular, the authors believe the following systems are the most promising:

1) alginate/cellulose sulfate//polymethylene-co-guanidine/calcium chloride;
2) alginate/cellulose sulfate//polyvinylamine/calcium chloride;
3) κ-carrageenan/hyaluronic acid//protamine sulfate/calcium chloride and potassium chloride;
4) κ-carrageenan/alginate//protamine sulfate/calcium chloride and potassium chloride;

5) gellan/alginate//protamine sulfate/calcium chloride;
6) LE-pectin/cellulose sulfate//polyamine, quart/calcium chloride;
7) LE-pectin/LTM-agarose//polybrene (or polyamine, quart.)/calcium chloride.

The only systems not listed in Tables 2–4 which are also likely to yield walled permeselective capsule are those based on polyvinylamine and chitosan. However, further research is required on the blending and processing of polyvinylamine systems, and the modification of chitosan, to enable the production of mechanically stable capsules which do not rupture catastrophically and slowly degrade as the present systems do under gelling with divalent ions.

A comparison of Tables 1 and 5 reveals the novelty of our multicomponent approach to capsule formation and permeability control. Of the existing chemistries in Table 5 only capsules #3, 4, and 5 conform to our recipe (technol-

Table 5. Literature data on polymer blends used for encapsulation

Polymer System	Polyanion Blend	Polycation Blend	Comment	Reference
1	CMC (HMW)/ Chondroitin Sulfate A	Chitosan	CMC offers water retention, CSA is the extracellular matrix component	17
2	CMC (HMW)/ Polygalacturonic Acid (Pectin)	Chitosan	CMC offers water retention	17
3	Alginate/Heparin	Protamine sulfate/ Polyethyleneimine/ Calcium Chloride	Heparin/Protamine sulfate form the primary complex polymer; alginate is the precasting polymer; protamine sulfate is the small polycation.	25
4	Alginate/ Polyvinylsulfate	Glycol-Chitosan Quaternary/ Calcium Chloride	Complex is formed between both alginate and polyvinylsulfate and chitosan; alginate is the precasting polymer.	26
5	Agarose/ Polystyrene-sulfonate	Polybrene	Polystyrene/Polybrene form the complex; agarose is the thermally gelled precast polymer; polybrene is the small polycation.	27
6	CMC/ Chondroitin Sulfate A	Polyethyleneimine	Only precast membrane on glass; CMC retains water.	21
7	Agarose/ Alginate	Dextran/Calcium Chloride	Agarose is the thermally gelled precast polmer; Dextran adjusts the viscosity.	10
8	Chitosan	Tripolyphosphate/ Dextran	Dextran adjusts the viscosity.	10
9	K Carrageenan	Polyamine/ Polyethyleneimine	Both polycations are small species.	28

CMC: Carboxymethylcellulose

ogy) and these were developed to improve the mechanical properties and stability of the standard APA system [9]. In particular our alginate/cellulose sulfate/polymethylene-*co*-guanidine/calcium chloride capsule has been shown to reverse diabetes in NOD mice (xenograph islets from rats) for period exceeding 120 days [23]. It is believed that this chemistry offers advantages to the APA capsule and it is being evaluated for other immunoisolation applications. The effect of particle size and the control of permeability are also under investigation in our laboratory.

Our screening and testing of multicomponent capsules/beads is incomplete. However, it offers a novel approach for the material selection for immobilization devices, which permits the simultaneous control of permeability, mechanical stability, and compatibility. The alternative multicomponent systems presented herein offer new possibilities for biomaterials, particularly those employed in bioartificial organs.

5 Conclusions

Based on a previous polymer and cytotoxicity screening [8], seven multicomponent polymer systems were identified as good candidates for further investigation. The capsule chemistry, reaction conditions, permeability, and transparency are shown in Tables 3 and 4 along with an overall ranking of each capsule as a prospective immunoisolation barrier. The single most important observation from these studies is the dominance of the diffusion of small molecular polycationic species and its importance in the build-up of microscopically visible permeability barrier (the so named "wall complex"). Based on the screening presented herein and in a preceding publications [8, 29], the following guidelines can be delineated for the selection of multicomponent polymer blends.

The inner (core) polymer blend should include:

a) one ionotropically gelling polyanion for precasting (e.g., alginate, κ-carrageenan); or
b) one thermosetting polyanion such as agarose, can also be used for precasting; or
c) an aqueous retainer, filler, or viscosity modifier. CMC or cellulose sulfate are examples. Chondroitin sulfate can be employed as a component of the extracellular matrix; and
d) one "wall-building" polyanion (e.g., hyaluronic acid, cellulose sulfate).

The outer (receiving bath) polymer should include:

a) a divalent or monovalent cation for ionotropic gelling, or
b) a low molecular weight polycation (typically a "wall-building" polymer such as polymethylene-*co*-guanidine or protamine sulfate), or
c) a mixture of low and high molecular weight polycations (e.g., spermine and polylysine).

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Controlled Synthesis of Polymers Using the Iniferter Technique: Developments in Living Radical Polymerization

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In this review, we describe the concept of iniferters and the model for living radical polymerization in a homogeneous system, which was proposed in 1982 by one of the authors to enable the controlled synthesis and molecular design of polymers through the radical polymerization process. The iniferters are classified into several types: thermal or photoiniferters; mono-, di-, tetra-, or polyfunctional iniferters; monomeric, polymeric, or gel iniferters; monomer or macromonomer-iniferters, leading to the syntheses of monofunctional, telechelic, and polyfunctional polymers, block and graft copolymers, and branched, star, and cross-linked polymers. Phenylazotriphenylmethane and tetraphenylethane derivatives serve as thermal iniferters, and some organic sulfur compounds act as photoiniferters. Among the iniferters, several compounds containing *N,N*-diethyldithiocarbamyl groups were found to be excellent for the synthesis of polymers with well-controlled structures. The synthesis of various types of block, star, and graft polymers with a controlled chain structure through living radical polymerization using dithiocarbamate compounds as photoiniferters is described. In the last section, the recent developments in living radical polymerization using nitroxides and transition-metal complexes since 1993 up to 1997 have also been reviewed.

Keywords: Controlled Polymerization; Living Radical Polymerization; Iniferter; Chain-End Structure; Molecular Weight Control; Block Copolymer; Dithiocarbamate; Disulfide; Nitroxide; Transition Metal Complex

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List of Symbols and Abbreviation

AA	acrylic acid
AIBN	2,2'-azobisisobutyronitrile
AN	acrylonitrile
BA	*n*-butyl acrylate
BD	butadiene
BPO	benzoyl peroxide
ClSt	*p*-chlorostyrene
C_{tr}	chain transfer constant
DC	dithiocarbamate
DiPF	diisopropyl fumarate
DMS	dimethylsiloxane
EA	ethyl acrylate
EMA	ethyl methacrylate
EO	ethylene oxide
IB	isobutene
IBVE	isobutyl vinyl ether
MA	methyl acrylate

MAn	maleic anhydride
MMA	methyl methacrylate
M_n	number-average molecular weight
MOSt	*p*-methoxystyrene
M_w	weight-average molecular weight
poly(SAN)	poly(styrene-*co*-acrylonitrile)
poly(VA)	poly(vinyl alcohol)
PSG	polystyrene gel
St	styrene
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy
VAc	vinyl acetate
VBCl	*p*-vinylbenzyl chloride
VCl	vinyl chloride

1 Introduction

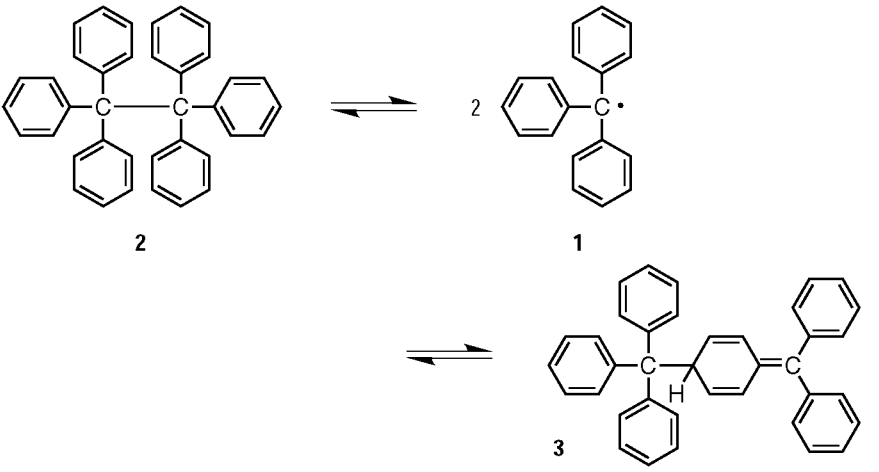
Radical polymerization is the most useful method for a large-scale preparation of various kinds of vinyl polymers. More than 70 % of vinyl polymers (i. e. more than 50 % of all plastics) are produced by the radical polymerization process industrially, because this method has a large number of advantages arising from the characteristics of intermediate free-radicals for vinyl polymer synthesis beyond ionic and coordination polymerizations, e. g., high polymerization and copolymerization reactivities of many varieties of vinyl monomers, especially of the monomers with polar and unprotected functional groups, a simple procedure for polymerizations, excellent reproducibility of the polymerization reaction due to tolerance to impurities, facile prediction of the polymerization reactions from the accumulated data of the elementary reaction mechanisms and of the monomer structure-reactivity relationships, utilization of water as a reaction medium, and so on.

However, radical polymerizations still have some unsolved problems, one of which is the control of the reactivities of monomers and the produced radicals therefrom in each elementary reaction, in other words the control of initiation, propagation, termination, and chain transfer reaction steps during polymerization. The manner and rate of these reactions determine the structure of the polymer chain produced. The control of the primary structure of polymers is now most important, e. g., molecular weight, molecular weight distribution, sequence distribution, stereoregularity, chain-end structures, and branching, because the formation of higher-order structures, physical properties, and various functions of polymers significantly depend on the primary chain structures.

Since the discovery of a living polymer in the anionic polymerization of St with a sodium/naphthalene initiator in 1956 by Szwarc [1,2], much effort has been made to find a living polymerization system to control the molecular weight, molecular weight distribution, and end groups of polymers. Thereafter, living polymerization systems [3] have been developed in the cationic [4–6], ring-opening [7], metathesis [8–10], coordination [11], group transfer [12], and immortal polymerizations [13], as well as the anionic polymerization of many kinds of monomers other than St [14, 15]. Some attempts have also been made to find a living radical polymerization sys-

tem. Because the propagating radical as an active species in radical polymerization is very short-lived in a homogeneous system, it is difficult to obtain the living (long-lived) propagating radical, except in the case in which its mobility markedly decreases. Therefore, various approaches have been made to construct the living systems through radical polymerization. The strategies and the detailed results by each researcher are summarized in recent reviews [3, 16–36].

Free radicals are classified from their lifetimes into long-lived (stable) and short-lived (intermediate) radicals. The most famous and first example of the former is triphenylmethyl (**1**), which was discovered just one hundred years ago by Gomberg [37].



2 **1** **3**

(1)

In the paper published in 1900, he reported that hexaphenylethane (**2**) existed in an equilibrium mixture with **1**. In 1968, the structure of the dimer of **1** was corrected to be 1-diphenylmethylene-4-triphenylmethyl-2,5-cyclohexadiene **3**, not **2** [38]. Since Gomberg's discovery, a number of stable radicals have been synthesized and characterized, e.g., triarylmethyls, phenoxyls, diphenylpicrylhydrazyl and its analogs, and nitroxides [39–43]. The radical **1** is stable, if oxygen, iodine, and other materials which react easily with it are absent. Such stable radicals scarcely initiate vinyl polymerization, but they easily combine with reactive (short-lived) propagating radicals to form non-paramagnetic compounds. Thus, these stable radicals have been used as radical scavengers or polymerization inhibitors in radical polymerization.

On the other hand, the presence of a short-lived free radical was confirmed first from an elegant experiment in 1929 by Paneth and Hofeditz [44], although the existence of paramagnetic species was pointed out in the middle of the 19th century by Faraday [45]. When tetramethyllead was thermolyzed, a methyl radical was postulated to be formed as the reaction intermediate (Eq. 2)

$$(CH_3)_4Pb \rightleftharpoons 4 \cdot CH_3 + Pb \qquad (2)$$

The methyl radical immediately reacts with a lead mirror to regenerate tetramethyllead or reacts with others to give ethane by combination, indicating that the methyl radical is very short lived, and the half-life was evaluated as 6×10^{-3} s. Similar short-lived radicals were assumed to have existed as a reaction intermediate (chain carrier) of chain reactions including the thermal polymerization of St by Staudinger and Frost [46] and Flory [47]. The concentrations of the primary, initiating, and propagating radicals are as low as 10^{-7}–10^{-9} mol/L or less during polymerization. Therefore, the detection of the radicals, as well as the determination of their concentration by electron spin resonance spectroscopy, are not easy for the polymerizations of common vinyl monomers, except for some cases when the polymerization proceeds at a high steady-state radical concentration as seen in the polymerizations of fumarates, itaconates, and maleimides with significantly bulky substituents [48, 49]. The propagating radicals are detectable without using special apparatus and technique when they are present in matrices, such as precipitated polymers, micro gels, microsphere, or glassy solids, in which their mobility markedly decreases [17, 50].

In 1957, Zimm and coworkers [51] reported the possibility for the preparation of monodisperse poly(St) by emulsion polymerization under the control of the number of polymer radicals in the micelle with intermittent irradiation of light. This idea was developed for other emulsion polymerizations using systems of ozonized polypropylene and some reducing agents as initiators, from which ultrahigh molecular weight poly(St) with polydispersities of 1.01–1.13 was produced [52, 53]. These polymerizations were further applied to the preparation of block copolymers. Other possibilities for preparing long-lived propagating radicals were also assumed for radical polymerizations of some organized monomers complexed with host compounds such as perhydrotriphenylene [54] and steroids [55, 56], of ethylene with the system triethylaluminum/γ-butyrolactone/*tert*-butyl perisobutyrate as the initiator [57], and of MMA in the presence of some metallic compounds such as zinc chloride [58] or system BPO/chromous acetate [59, 60] as well as in viscous phosphoric acid [61, 62]. These long-lived radicals were also used for the preparation of block copolymers, but no clear evidence for living radical polymerization was given in these papers.

We can classify the approaches to stabilizing propagating radicals into physical and chemical processes. In the physical approach, the distinct diffusion rate of the polymer radicals from that of the monomers might be a key for the design of the system. If the termination between macromolecular radicals is ultimately suppressed, but the propagation reaction occurs on account of the fast diffusion of a small molecule, the living polymerization would be practical. Propagation could be preferential to termination only when the radical concentration is extremely low because a radical-radical reaction readily occurs compared with a radical addition and its reaction rate is proportional to the square of the radical concentration. Matrix polymerization and inclusion polymerization are expected for this purpose [56, 63], but the fine architecture of the matrices and host compounds is indispensable and it is not easy. Simple chemical stabilization of the polymer radical, e.g., by complex formation, would decrease not only the rate of termination, but also the propagation. Therefore, for the design of a liv-

ing radical polymerization, the temporary stabilization of the propagating radicals is necessary, as shown in Eq. (3).

$$\underset{\text{active species (propagating radical)}}{P\bullet} \underset{-X^*}{\overset{X^*}{\rightleftharpoons}} \underset{\text{dormant species}}{P\text{-}X} \qquad (3)$$

From 1957 to 1960, Otsu and coworkers reported that tetraethylthiuram disulfide (see **13**) photochemically or thermally induced the radical polymerization of St and MMA to yield relatively low-molecular-weight polymers having two initiator fragments in both chain ends (see Eq. 14), although the polymerization reactivities (rates) were relatively low. A kinetic study confirmed that primary radical termination occurred during these polymerizations. Moreover, it was found that the polymers having the initiator fragments at the chain ends induced further radical polymerization of second monomers under the irradiation of light to give block copolymers. From these results, Otsu and Yoshida proposed in 1982 the new concept of iniferter [64] and simultaneously the model for a living radical polymerization in a homogeneous system [16].

As previously described, it is very difficult to realize living radical polymerization in a homogeneous system, so that the introduction of some novel ideas are indispensable, different from living ionic polymerizations. Namely, (i) a short-lived propagating radical temporarily exists through stable covalent-bond formation, which (ii) can dissociate again into the propagating radical and a less or non-reactive small radical, and (iii) the former radical reacts with a monomer and then terminates with the latter radical to give an identical covalent-bond compound, which is considered to be a dormant species of the propagating radical. To design the living radical polymerization systems, the choice of the group (or atom) X in Eq. (3), e.g., stable radicals, halogen atoms, or transition metals, and the control of the equilibrium between the active and dormant species are the most important.

In this article these concepts are described first, and the results of the controlled synthesis of polymers using the iniferter technique are discussed. In the last section, the excellent progress of living radical polymerization during recent years, from 1993 up to 1997, is summarized.

2
Iniferter and Iniferter Technique

2.1
Definition of Iniferter

The polymer formation in the radical polymerization of vinyl monomers initiated by a usual initiator R-R is expressed by Eqs. (4) and (5) if termination proceeds via combination and disproportionation and no chain transfer reaction occurs.

$$R\text{–}R + 2n\,M \longrightarrow R\text{–}(M)_{2n}\text{–}R \quad (4)$$

$$R\text{–}R + 2n\,M \longrightarrow R\text{–}(M)_{n-1}\text{–}M\ (-H) + R\text{–}(M)_{n-1}\text{–}M\ (+H) \quad (5)$$

When the termination involves only combination, the polymerization gives a polymer with two initiator fragments at its chain ends. Because termination in the bulk polymerization of St with AIBN at a moderate temperature occurs by combination, the polymer obtained has two initiator fragments at both chain ends. In the radical polymerization of most monomers, however, termination by disproportionation and chain transfer reactions occur; it is therefore impossible to control these termination reactions, i.e., the chain-end structure. Therefore, the number of initiator fragments per one molecule is always less than two.

The molecular weight and chain-end structure of polymers can be modified using the chain transfer reaction [65–68]. When an appropriate chain transfer agent, X-Y, is used in radical polymerization, two types of oligomers or telomers having different end groups, **4** and **5**, are formed depending on the value of the chain transfer constant, C_{tr}, of X-Y used.

$$X\text{–}Y + n\,M \xrightarrow{R^{\bullet}} \underset{\mathbf{4}}{R\text{–}(M)_n\text{–}Y} + \underset{\mathbf{5}}{X\text{–}(M)_n\text{–}Y} \quad (6)$$

If C_{tr} is very large, telomer **5** bearing X and Y groups at both chain ends is expected to be mainly formed, and a very small amount of **4** is also formed. When the telomer is of quite low molecular weight, **4** might be separated from the mixture by distillation, similar to telomers obtained for ethylene and carbon tetrachloride. However, if chain transfer agents having relatively small C_{tr} are used, the amount of the oligomer **5** decreases, and that of oligomer **4** increases. Therefore, the end structure of these oligomers cannot be controlled strictly by this method.

If we use initiators R-R' which have very high reactivities for the chain transfer reaction to the initiator and/or primary radical termination, i.e., ordinary bimolecular termination is neglected, it is expected that a polymer will be obtained with two initiator fragments at the chain ends (Eq. 7):

$$R\text{–}R' + n\,M \longrightarrow R\text{–}(M)_n\text{–}R' \quad (7)$$

These radical polymerizations may simply be considered as an insertion of monomer molecules into the R-R' bond of the initiator leading to the polymer with two initiator fragments. Thus, the end groups of the polymer are controlled by the initiator used. Otsu proposed the name "iniferter" (*ini*tiator-trans*fer* agent-*ter*minator) for the initiators with such functions [64]. Many radical initiators, such as peroxides, azo compounds, tetraphenylethane derivatives, and organic sulfur compounds, may be expected to serve as an iniferter, if monomers and polymerization conditions are selected. Some peroxides show relatively high

chain transfer reactivities to yield a bifunctional oligomer or polymers. Aliphatic azo compounds show no chain transfer reactivity, but, importantly, in the polymerization with some tetraphenyl and unsymmetric azo compounds, primary radical termination occurs. Tetraphenylethane derivatives produce diphenylmethyl radicals which can participate in both initiation and termination. Organic sulfur compounds, such as alkyl or aryl sulfides and disulfides, have high chain transfer reactivity, and part of the thiyl radicals produced may undergo primary radical termination because they are not so reactive for initiation. From the viewpoint of the tailor-made polymer synthesis, iniferters having the *N,N*-diethyldithiocarbamyl (DC) group were found to be excellent, as is shown in Sect. 5.

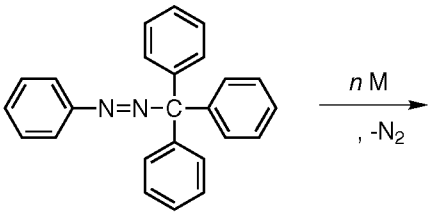
2.2 Classification of Iniferters

There are two types of A-B and B-B type iniferters. A-B type iniferters thermally or photochemically dissociate into different radicals (Eq. 8):

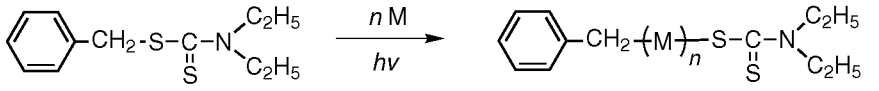
$$A\text{–}B \xrightarrow{\text{or } h\nu} A\bullet + B\bullet \qquad (8)$$

where A• is the reactive radical, which participates only in initiation, and B• is the less or non-reactive radical which cannot enter initiation and acts as a primary radical terminator. Phenylazotriphenylmethane (**6**), which is often used as a source of the phenyl radical, benzyl *N,N*-diethyldithiocarbamate (**7**) and *p*-xylylene bis(*N,N*-diethyldithiocarbamate) (**8**) are included in the A-B type iniferters.

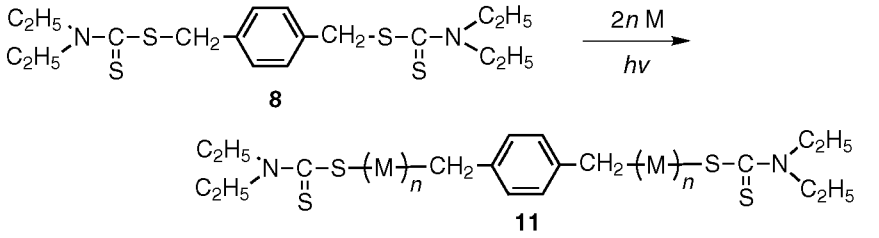
These iniferters thermally or photochemically dissociate at the weak bonds, and then monomer molecules are inserted by propagation, followed by primary radical termination and/or chain transfer to give polymers (**9**–**11**) (Eqs. 9–11), which also contain iniferter bonds at the chain ends. These polymers may further act as the polymeric iniferters.



6 **9** (9)



7 **10** (10)

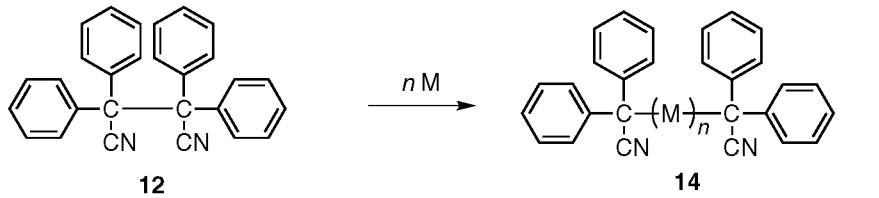


(11)

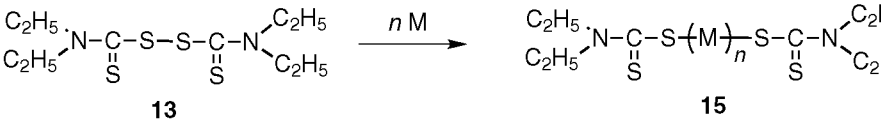
On the other hand, the B-B type iniferters dissociate into two identical radicals as follows (Eq. 12):

(12)

where B• is the less reactive radical which can enter into both initiation and primary radical termination. In the B-B type iniferters, tetraphenylsuccinodinitrile (**12**) and tetraethylthiuram disulfide (**13**) are involved, and they provide polymers (**14** and **15**) having iniferter fragments at both chain ends, i.e., polymeric iniferters (Eqs. 13 and 14).



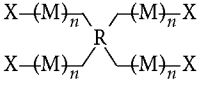
(13)



(14)

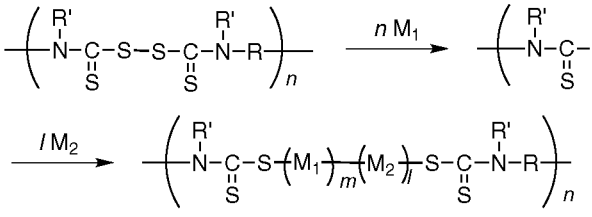
Polymers **14** and **15** seem to serve as bifunctional iniferters from their structures, but they eventually act as the monofunctional iniferters because of the difference in the chain-end structure (see Sect. 4 for details).

Table 1. Classification and Applications of Iniferters with the Dithiocarbamate Group

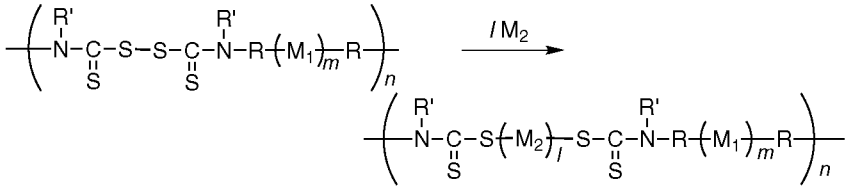
Iniferter	Structure and Reaction			Application to Polymer Synthesis
	Iniferter		Polymeric iniferter	
Monofunctional Iniferter	R-X	⟶	R–(M)$_n$–X	End-functional polymer, AB-type block copolymer
Difunctional Iniferter	X-X	⟶	X–(M)$_n$–X	Telechelic polymer, AB- or ABA-type block copolymer
	X-R-X	⟶	X–(M)$_n$–R–(M)$_n$–X	Telechelic polymer, ABA-type block copolymer
Tetrafunctional Iniferter		⟶		Star polymer, Star block copolymer, Cross-linked polymer
Polyfunctional Iniferter	–(R-X)$_m$–	⟶	–(R–(M)$_n$–X)$_m$–	Multiblock copolymer
Monomer Iniferter	C=C-R-X	⟶	–(C-C)$_n$–(M)$_m$– R–(M)$_l$–X	Comb-like polymer, Graft copolymer
		⟶	C=C-R–(M)$_l$–X	Macromonomer
Gel Iniferter	Gel-R-X	⟶	Gel-R–(M)$_n$–X	Multiblock copolymer

X: Dithiocarbamate (DC) group.

The A-B type iniferters are more useful than the B-B type for the more efficient synthesis of polymers with controlled structure: The functionality of the iniferters can be controlled by changing the number of the A-B bond introduced into an iniferter molecule, for example, B-A-B as the bifunctional iniferter. Detailed classification and application of the iniferters having DC groups are summarized in Table 1. In Eqs. (9)–(11), **6** and **7** serve as the monofunctional iniferters, **9** and **10** as the monofunctional polymeric iniferters, and **8** and **11** as the bifunctional iniferters. Tetrafunctional and polyfunctional iniferters and gel-iniferters are used for the synthesis of star polymers, graft copolymers, and multiblock copolymers, respectively (see Sect. 5). When a polymer implying DC moieties in the main chain is used, a multifunctional polymeric iniferter can be prepared (Eqs. 15 and 16), which is further applied to the synthesis of multiblock copolymers.

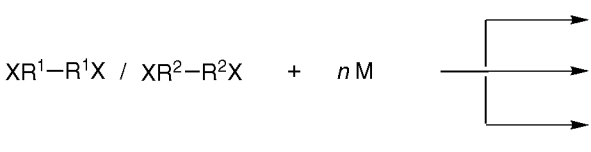


(15)



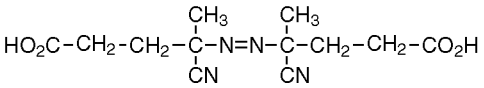
(16)

Otsu and coworkers subsequently proposed a two-component iniferter system [69], the combined use of an iniferter and a chain transfer agent to achieve the control of the chain-end structure of a wider variety of polymers when it is difficult to control the structure using only a single compound. This system consists of two compounds bearing the same functional group, one being an initiator or an iniferter (XR^1-R^1X), and the other a chain transfer agent (XR^2-R^2X). When radical polymerization is carried out in the presence of these compounds, polymers having three types of end groups are produced as shown in Eq. (17)



(17)

The resulting polymers always have the same functional group X at both chain ends. Therefore, telechelic polymers can be readily synthesized by the two-component iniferter system. An example is the polymerization of several monomers with 4,4'-azobiscyanovaleric acid (**16**) and dithiodiglycolic acid (**17**) as the initiator and the chain transfer agent, respectively, to synthesize the polymers having carboxyl groups at both chain ends [69].



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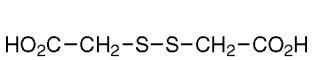


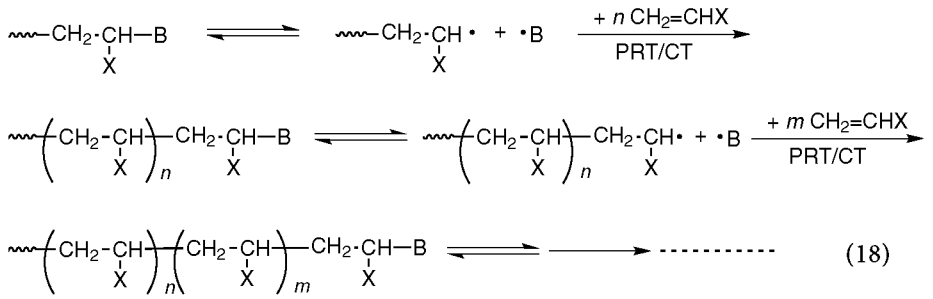
17

Such a two-component iniferter technique is also applied to the living radical polymerization of several DC photoiniferters for the design of block and graft copolymer synthesis (Sect. 5).

3 A Model for Living Radical Polymerization in a Homogeneous System

In 1957, Otsu and coworkers reported that the polymer obtained from St with **13** could induce the radical polymerization of second monomers leading to block copolymers [70–74]. Poly(St)-*block*-poly(MMA), poly(St)-*block*-poly (AN), poly(St)-*block*-poly(VAc), and poly(St)-*block*-poly(VA) were prepared from the end-functional poly(St) [75]. In the photopolymerization of St and MMA with **13**, it was also confirmed that the molecular weight of the polymers produced linearly increased with the reaction time, although the reaction mechanism was not ascertained at that time. Thereafter, the poly(St) produced with **13** was confirmed to have two DC end groups, which can further dissociate photochemically [76].

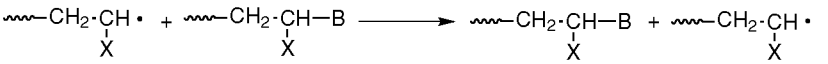
When the end groups of the polymers obtained by radical polymerization using certain iniferters still have an iniferter function, such radical polymerization is expected to proceed via a living radical mechanism even in a homogeneous system, i.e., both the yield and the molecular weight of the polymers produced increase with reaction time. The generalized model is shown in Eq. (18) [16]:



(18)

PRT: primary radical termination, CT: chain transfer

The C-B bond, which acts as the iniferter, in the propagating chain end thermally or photochemically dissociates into a reactive propagating radical and a less or non-reactive small radical which does not enter the initiation of a new polymer chain, but readily undergoes primary radical termination with a propagating radical to reproduce the identical C-B bond. Any chain transfer reaction of the propagating radical to the C-B bond would give a similar propagating radical and the C-B bond (Eq. 19):

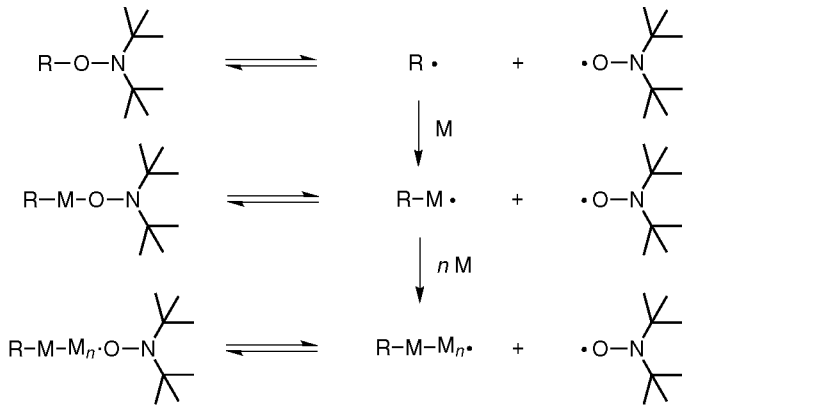


(19)

When the polymerization proceeds via the repetition of the dissociation at the C-B bond, the addition of monomers to the propagating radical, and primary radical termination with B• and/or of chain transfer of the propagating radical to the C-B bond, such polymerization may proceed via a living radical mechanism. As an extreme case, if the polymerization proceeds via a stepwise insertion of one monomer molecule into the C-B bond, it would result in a successive reaction.

The polymerization of St and MMA with some sulfur compounds, especially containing DC groups as the photoiniferter, and of MMA with **6** as the thermal iniferter were found to proceed via a mechanism close to the model of the living radical polymerization of Eq. (18). The resulting polymeric iniferters were used for block copolymer synthesis. These polymerizations show somewhat different features from the other living polymerizations, such as living anionic polymerization, because the polymerization proceeds via a free-radical chain reaction mechanism. For example, the stereoregularity of the polymers obtained is the same as that of ordinary free-radical polymerization because of the free propagation. Polymerization kinetics for the radical polymerization using iniferters have been discussed in several articles [21, 22, 25, 77], but they are not dealt with in this review.

In 1984, Solomon et al. [78–80] also independently reported that some alkoxyamines and related compounds induced living radical polymerization (Eq. 20), being similar to Eq. (18).



(20)

The resulting polymers can further induce the radical polymerization of second monomers to give block copolymers. The polymerization with the alkoxyamines has developed to the recent living radical polymerization providing polymers with well-controlled molecular weight and molecular weight distribution, as will be described in Sect 6.1.

4
Control of the Chain-End Structure of Polymers with the Iniferter Technique and Feature of the Living Radical Polymerization

4.1
Polymerization with Thermal Iniferters

4.1.1
Phenylazotriphenylmethane

The results of the radical polymerization of MMA in bulk at 60–100 °C with **6** as the A-B type thermal iniferter are shown in Fig. 1 [16, 81]. In this polymerization, the molecular weight of the polymers produced increased with the reaction time.

Phenyl and triphenylmethyl radicals generated from **6** contribute to the initiation and the termination, respectively, resulting in polymer **18** because of the remarkably different reactivities of these radicals (Eq. 21). The ω-chain end terminated with **1** thermally redissociates to induce further polymerization. Therefore, the polymerization proceeded via a mechanism close to the model in Eq. (18). The recombination product of methyl isobutyryl radical and **1** was reported to have a quinonoide structure [82], suggesting a similar structure of the chain end, **18b**.

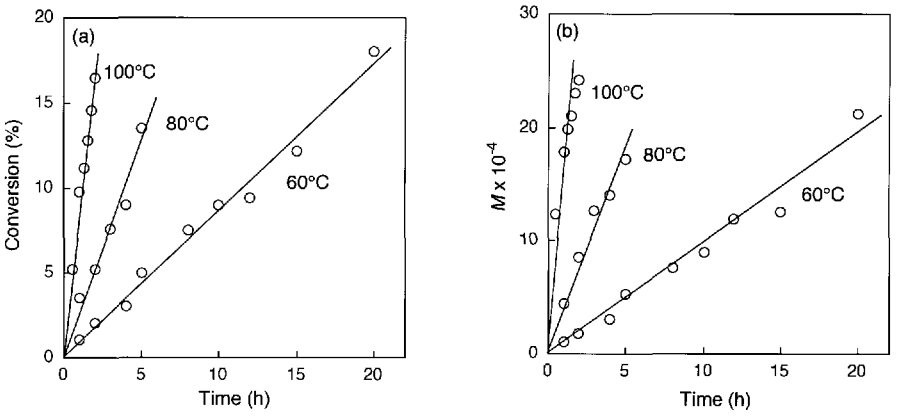
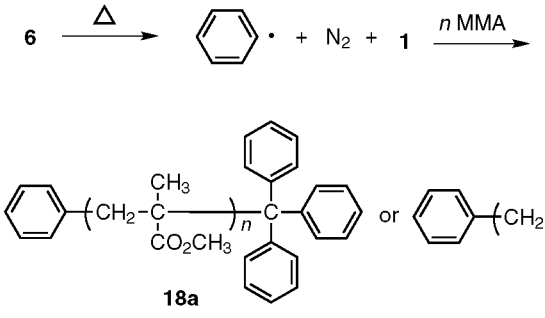
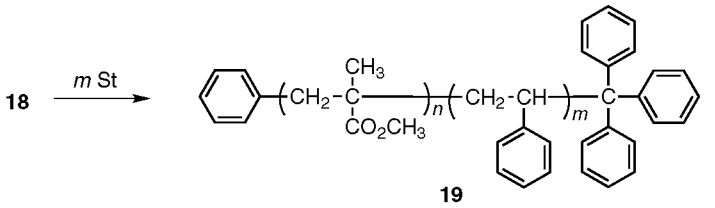


Fig. 1. Time-conversion **(a)** and time-molecular weight **(b)** relationships for bulk polymerization of MMA with **6** at 60-100 °C. [6] = 1.0 x 10^{-2} mol/L.



(21)

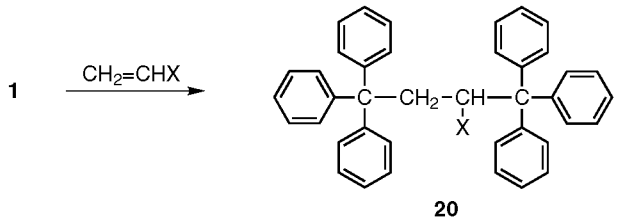
Block copolymerization was carried out in the bulk polymerization of St using **18** as the polymeric iniferter. The block copolymer was isolated with 63–72 % yield by solvent extraction. In contrast with the polymerization of MMA with **6**, the St polymerization with **18** as the polymeric iniferter does not proceed via the living radical polymerization mechanism, because the ω-chain end of the block copolymer **19** in Eq. (22) has the penta-substituted ethane structure, of which the C-C bond will dissociate less frequently than the C-C bond of hexa-substituted ethanes, e.g., the ω-chain end of **18**. This result agrees with the fact that the polymerization of St with **6** does not proceed through a living radical polymerization mechanism. Therefore, **18** is suitably used for the block copolymerization of 1,1-diubstituted ethylenes such as methacrylonitrile and alkyl methacrylates [83].



(22)

4.1.2 *Tetraphenylethane Derivatives*

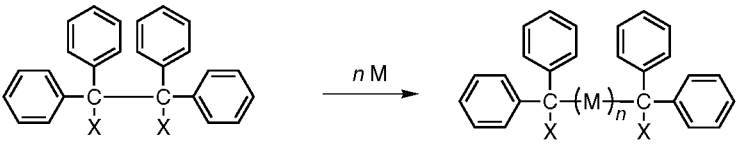
Stable radicals such as **1** are commonly used as the radical trapping agents and inhibitors or modifiers for polymerization. In the reaction of **1** with vinyl monomers, such as St, VAc, and BD, the adducts **20** are isolated (Eq. 23):



(23)

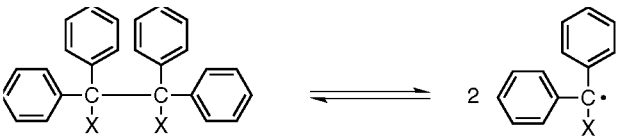
Here the radical **1** acts as a strong terminator to prevent the formation of oligomers and polymers. On the other hand, it is expected that the substituted diphenylmethyl radicals which are less stable than **1** serve as both initiators and primary radical terminators. In fact, it was reported [84] that the apparent polymerization reactivities decreased in the following order: diphenylmethyl, phenylmethyl, and triphenylmethyl radicals, which were derived from the initiator systems consisting of arylmethyl halides and silver.

In the polymerization with tetraphenylethanes as the initiators, the polymer produced would be obtained as shown in Eq. (24) because the generated diphenylmethyl radical can function as both an initiator and a terminator.



(24)

1,1,2,2-Tetraphenylethane (**21a**) scarcely dissociates into a radical under polymerization conditions because of its large bond dissociation energy, but when both hydrogens attached to the carbon atoms in **21a** are replaced by other groups, it may easily dissociate into radicals and give an equilibrium mixture [85–87]. The structure of the dimer of **22**, i.e., ethane-type dimers **21** or quinonoide-type dimers as an analog of **3**, depends on the substituents X or the substituents on the phenyl groups. The dissociation of **21** has been investigated by ESR spectroscopy [87–91].



21 **22**

(25)

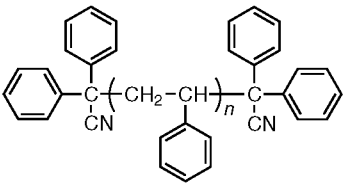
X = H (**21a**), C_2H_5 (**21b**), -C_2H_4- (**21c**), OC_6H_5 (**21d**), $OSi(CH_3)_3$ (**21e**), OH (**21f**)
$OCOCH_3$ (**21g**), OCH_3 (**21h**), CH_3 (**21i**), $CO_2C_2H_5$ (**21j**), CN (**12**)

In 1939, Schulz [92–94] first reported that **12** (X=CN in **21**) served as an initiator for the radical polymerization of MMA and St. Thereafter, Hey and Misra [95] also reported the polymerization of St with **12** or its *p*-methoxy substituted derivatives. Borsig et al. [96, 97] reported in 1967 the polymerization of MMA and St with 3,3,4,4-tetraphenylcyclohexane (**21b**) and 1,1,2,2-tetraphenylcyclopentane (**21c**) and that the reaction orders of the polymerization rates with respect to the concentrations of **21b** and **21c** were 0.25 and 0.20, respectively, and concluded that the primary radical termination predominantly occurred. It was noted that in these polymerizations the average molecular weight of the polymer increased as a function of the polymerization time, although the clear reason was not described in these papers. It was also reported by the same authors that the resulting polymer could further induce block copolymerization [98].

In 1981, Braun and coworkers [99–103] systematically studied the polymerization of MMA with 1,2-diphenoxy-1,1,2,2-tetraphenylethane (**21d**) and asserted that the polymerization with **21d** proceeded via three steps: (i) primary radical termination to form an oligomer, (ii) a cleavage of the C-C bond at the oligomer chain end to form macro- and small radicals, and (iii) normal propagation and termination with an increase in the conversion after the consumption of the initiator radicals. The oligomers were separated by chromatography, and their structures were examined by NMR spectroscopy. Because these oligomers contained further dissociability at the chain-end group, they could initiate the polymerization of MMA and yielded block copolymers with other vinyl monomers [104]. Studies of polymerization with **12**, **21d**, and 1,2-bis(trimethylsiloxy)-1,1,2,2-tetraphenylethane (**21e**) have also been reported [104–110].

Some polymers with initiator fragments at the chain ends, which are produced by initiation and primary radical termination by the radical **22**, are expected to further act as iniferters; therefore, they are classified as polymeric iniferters (Eq. 24). When the relationship between the conversion and the molecular weight of the polymer for the radical polymerization of MMA with **12** as the iniferter was investigated, an increase in the molecular weight of the polymer depending on the conversion was observed, but the linear relationship between the polymerization time and the molecular weight possessed an intercept, i.e., the line did not pass through the origin, and the degree of the increase in the molecular weight is not so high [105, 106]. The dissociation of **12** results in the formation of the two radicals with an identical structure, one of which should take part in the initiation, and another should terminate the chain propagation in order to function as an effective iniferter. However, the radical actually tends to initiate the propagation of a new polymer chain rather than its termination. It was also found that poly(MMA) which was prepared with **12** gave a block copolymer with St, but block efficiency was not high [106].

In the polymerization of St, it was found that **12** scarcely induces living radical polymerization [111], because the C-C bond of the ω-chain end is a pentasubstituted ethane structure (**23**), while the ω-chain end of the polymer produced from the polymerization of MMA is a dissociable hexasubstituted ethane structure (**24**). The non-dissociation properties of the ω-chain end of the polymer produced in the St polymerization were also reported by Braun et al. [109, 112–116]. Namely, the St polymerization with **12** was a dead-end type polymerization. The dissociation of the chain ends was also examined by the experiments using the oligomer (n=1–3 in **24**) [117, 118] or a model compound of the chain-end structures, **25** [119]. The C-C bond length at the ω-chain end is 1.628 Å for **24** (n=1), which is longer than the ordinary C-C bonds [118].



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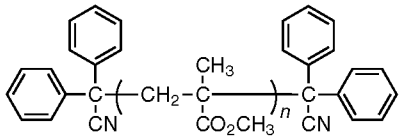

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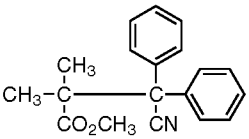
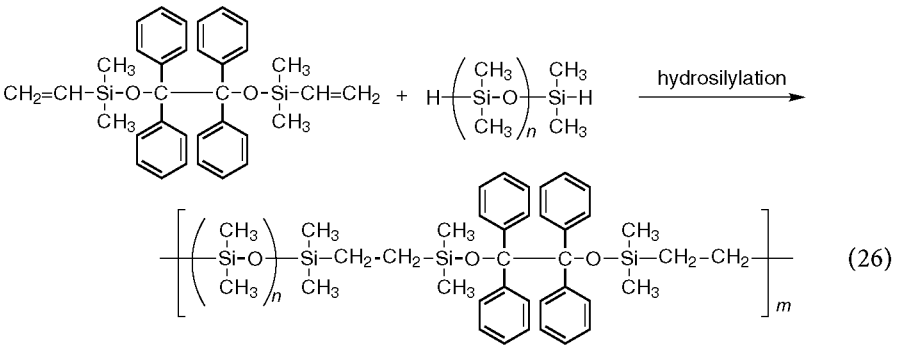


25

1,2-Dihydroxy-1,1,2,2-tetraphenylethane (**21f**) dissociates into radicals, but the degree of dissociation is low, for example, less than 5% at 120 °C [120]. The radicals generated from **21f** undergo disproportionation to give benzophenone and diphenylhydroxymethane. A hydrogen transfer of the radical produced from **21f** to a monomer causes the initiation of polymerization at high efficiency [103]. Radical dissociation of the bond including a diphenylhydroxymethyl moiety introduced surface of polymeric materials was applied to graft polymerization [121]. The disproportionation also easily occurs for **21b** and 2,2,3,3-tetraphenylbutane (**21i**). The ratios of the dissociation rate to the disproportionation rate were determined to be 7.4 and 101.4 for **21i** and **21b**, respectively [122]. On the other hand, 1,1,2,2-tetraphenyl-1,2-diacetoxyethane (**21g**) and 1,2-dimethoxy-1,1,2,2-tetraphenylethane (**21h**) may be used as the initiators because of no hydrogen transfer. It was also reported that diethyl 3,3,4,4-tetraphenylsuccinate (**21j**) easily dissociates into radicals [123].

Crivello et al. synthesized block copolymers consisting of poly(DMS) and vinyl polymer sequences to modify the mechanical properties and solvent resistance of poly(DMS). They used tetraphenylethane derivatives incorporated into the poly(DMS) chain through hydrosilylation (Eq. 26) [124–126]:

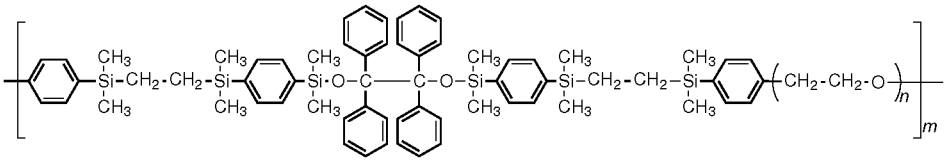


(26)

26

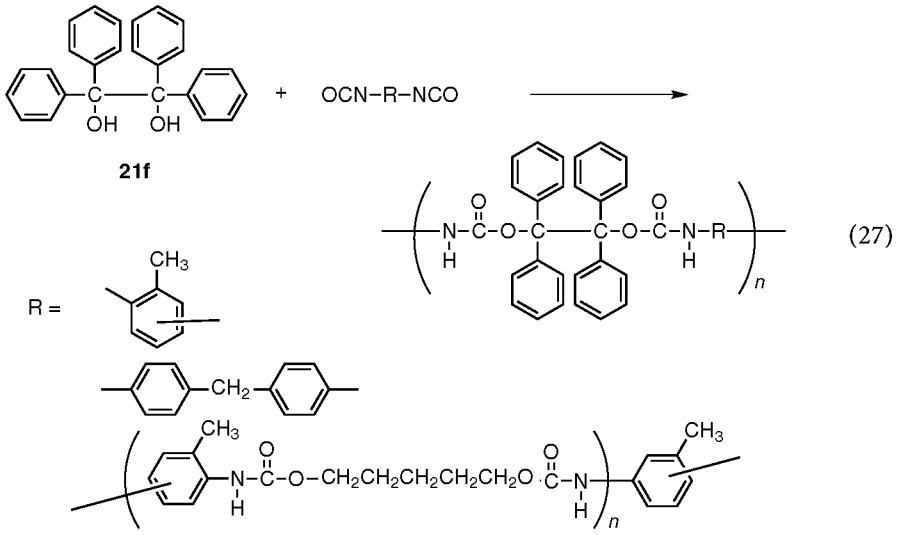
In the polymerization of MMA with **26**, the molecular weight of the resulting copolymer increased with the polymerization time (conversion). The St polymerization provided a multiblock copolymer by recombination. It was revealed that the length of the poly(St) segment as well as the mechanical properties of the block copolymer depended on the chain length of the poly(DMS) segments because of phase separation [127].

Santos et al. [128, 129] and Guerrero et al. [130] prepared segmented poly(EO) containing bistrialkylbenzopinacolate moieties to synthesize poly(St)-poly(EO) block copolymers. The St polymerization with the polymeric iniferter **27** was comparable to that initiated with small molecular benzopinacolates.



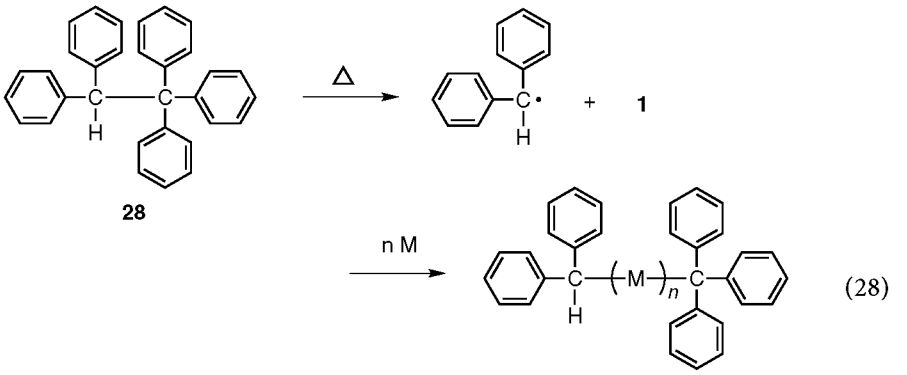
27

Polymeric iniferters synthesized from diisocyanates and **21f**, as shown in Eq. (27), were used to polymerize vinyl monomers, e.g., St, MMA, AN, and VBCl [131–137]. The multiblock copolymers of polyurethane and vinyl polymers were also characterized.



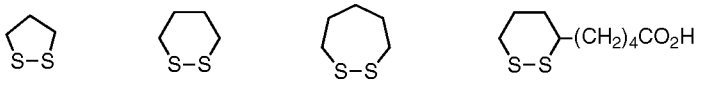
(27)

The tetraphenylethanes described above are symmetrical compounds used to generate the same two radicals by dissociation, while pentaphenylethane (**28**) is an unsymmetrical derivative, giving two different radicals, triphenylmethyl and diphenylmethyl radicals [138]. The former cannot initiate radical polymerization, but the latter is available as an initiating radical to produce the polymer **28**, which can function as the polymeric iniferter [106].



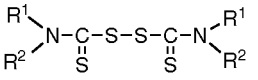
4.1.3 *Disulfides*

Dialkyl or diphenyl disulfides have been used as the initiators for polymerizations, and some disulfides also function as the terminator or the chain transfer agent. Recently, Endo found ring-enlargement polymerization of some cyclic alkylene disulfides (**29a–c**) as well as lipoic acid (**29d**) in a radical mechanism [139]. He also reported [140] in 1992 that these disulfides could induce the living radical polymerization of St at 120 °C. The molecular weight of the polymers produced linearly increased with the conversion, and no significant change in the molecular weight distribution was observed. The number of the disulfide units per polymer chain was confirmed to be constant as unity. The poly(St) obtained could further initiate the polymerization of MMA to give a block copolymer in high yield [141].

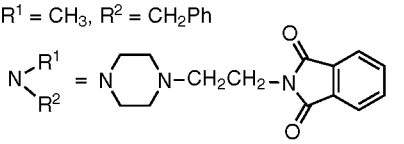


29a **29b** **29c** **29d**

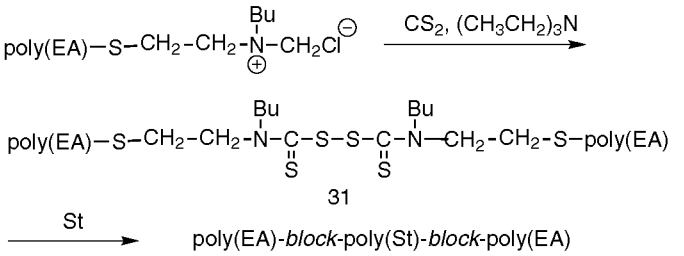
Nair et al. studied the kinetics of the polymerization of MMA at 60–95 °C using *N,N'*-diethyl-*N,N'*-di(hydroxyethyl)thiuram disulfide (**30a**) as the thermal iniferter [142]. The dependence of the iniferter concentration on the polymerization rate was examined. The chain transfer constant of the propagating radical of MMA to **30a** was determined to be 0.23–0.46 at 60–95 °C, resulting in the activation energy of 37.6 kJ/mol for the chain transfer. Other derivatives **30b–30d** were also prepared and used to derive telechelic polymers with the terminal phosphorus, amino, and other functional aromatic groups [143–145]. Thermal polymerization was also investigated with the end-functional poly(St) and poly(MMA) which were prepared using the iniferter **13** [146].



30

30a $R^1 = CH_2CH_3$, $R^2 = CH_2CH_2OH$
30b $R^1 = CH_3$, $R^2 = CH_2CH_2N(CH_3)P(=O)(OCH_2CH_3)_2$
30c $R^1 = CH_3$, $R^2 = CH_2Ph$
30d 

The amine-terminated poly(EA) was prepared by the chain transfer polymerization of EA in the presence of the salt of aminomercaptan, followed by the reaction with carbon disulfide to give the polymeric iniferter **31**. The polymerizations of St and MMA with **31** provided the triblock copolymers, poly(EA)-*block*-poly(St)-*block*-poly(EA) and poly(EA)-*block*-poly(MMA)-*block*-poly(EA), respectively, as shown in Eq. (29) [147]:

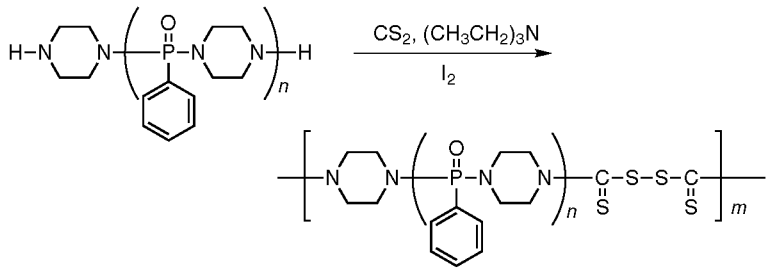


31

(29)

Similarly, various polymeric iniferters were applied to the syntheses of triblock copolymers with various sequences other than poly(EA), for example, poly(EO) [148], poly(AA), and poly(St) [25].

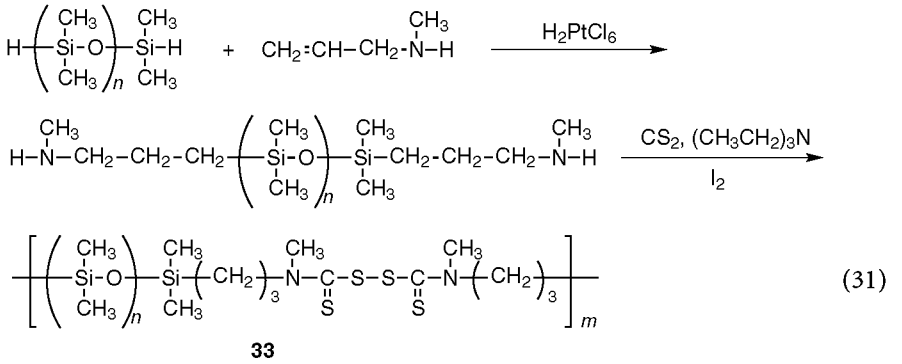
They also synthesized polymeric iniferters containing the disulfide moiety in the main chain [149, 150]. As shown in Eq. (30), polyphosphonamide, which was prepared by the polycondensation reaction of phenyl phosphoric dichloride with piperadine, was allowed to react with carbon disulfide in the presence of triethylamine, followed by oxidative coupling to yield the polymeric iniferter **32**. These polymeric iniferters were used for the synthesis of block copolymers with St or MMA, with the composition and block lengths controlled by the ratio of the concentration of the polymeric iniferter to the monomer or by conversion. The block copolymers of polyphosphonamide with poly(St) or poly(MMA) were found to have improved flame resistance characteristics.



32

(30)

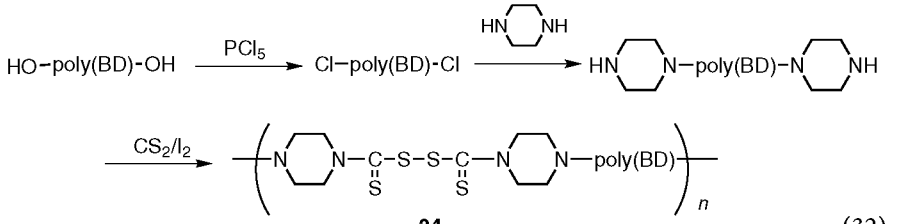
The polymeric disulfide iniferter consisting of poly(DMS) **33** was also similarly prepared (Eq. 31) [143, 151]. Block copolymers of poly(DMS) with MMA or St were synthesized with from two to eight blocks of both sequences per chain.



(31)

33

Recently, Kroeze et al. prepared polymeric iniferter **34** including poly(BD) segments in the main chain [152]. They successfully synthesized poly(BD)-*block*-poly(SAN), which was characterized by gel permeation chromatography, elemental analysis, thermogravimetric analysis, NMR, dynamic mechanical thermal analysis, and transmission electron microscopy. By varying the polymerization time and iniferter concentration, the composition and the sequence length were controlled. The analysis confirmed the chain microphase separation in the multiblock copolymers.



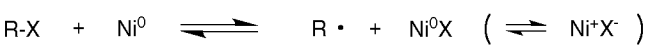
(32)

34

4.1.4 *Redox Iniferter*

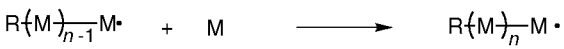
Because the polymerization with the thermal iniferters previously described was performed at a high temperature, some side reactions might be unavoidable, e.g., ordinary bimolecular termination between polymer radicals, disproportionation between a polymer radical and a small radical leading to deactivation of the iniferter site, initiation by the radical generated from the iniferter sites, rearrangements of the structure of the iniferter sites, and spontaneous initiation of polymerization.

To develop thermal iniferters which act at a lower temperature, Otsu and Tazaki proposed a redox iniferter system [153, 154]. For example, reduced nickel (Ni^0) reacts with organic halides (R-X) such as benzyl chloride to form a radical, which can initiate polymerization (Eqs. 33–35):



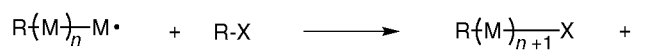
(33)

(34)



(35)

When R-X and NiX have high reactivities for chain transfer and/or primary radical termination (Eqs. 36 and 37), and the C-X bond at the chain end further reacts with Ni^0 by redox reaction (Eq. 38), the polymerization proceeds via a living radical polymerization mechanism. In this polymerization, the polymerization which has R and X groups at both chain ends is produced:



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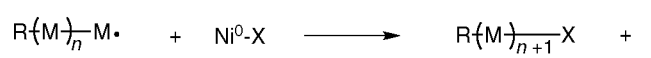


(37)

(38)

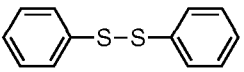
Similarly, it was also found that radical polymerization was induced in the $Ni(CO)_3(PPh_3)/CBrCl_3$ redox system [155]. This complex is soluble in the polymerization medium, and the polymerization proceeded in a homogeneous system. This redox iniferter system has been intensively developed to the recent successful living radical polymerization using transition-metal complexes in combination with alkyl halides by several independent research groups (see Sect. 6.2).

4.2 Polymerization with Photoiniferters

4.2.1 *Disulfides*

In polymerization with the compounds having a photodissociable DC group as photoiniferters, the polymerization can be performed at low temperature, such as room temperature, in contrast with thermal iniferters. Moreover, we can readily prepare many kinds of DC derivatives with various structures, indicating that the functionalization and molecular structure design are easy [156].

Tetraethylthiuram disulfide (**13**) induces St polymerization by the photodissociation of its S-S bond to give the polymer with C-S bonds at both chain ends (**15**). The C-S bond further acts as a polymeric photoiniferter, resulting in living radical polymerization. Eventually, some di- or monosulfides, as well as **13**, were also examined as photoiniferters and were found to induce polymerization via a living radical polymerization mechanism close to the model in Eq. (18), e.g., the polymerization of St with **35** and **36** [76, 157]. These disulfides were used for block copolymer synthesis [75, 157–161]:



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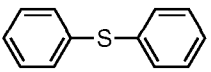


36

Figure 2 shows the time-conversion and time-molecular weight relationships in the photopolymerization of St and MMA with **13** at 30°C [16, 76, 157]. The yields and molecular weight of the polymer increased with polymerization time. From the analysis of the end groups of the polymer chain, it was confirmed that the number of the DC groups remained at two during polymerization (Table 2) [76, 156].

It was confirmed that the resulting polymers obtained from the St polymerization with **13** induced further photopolymerization of MMA to produce a block copolymer, and the yield and molecular weight increased as a function of the polymerization time, similar to the results for the polymerization of MMA with **13**, indicating that this block copolymerization also proceeds via a living radical polymerization mechanism [64]. Similar results were also obtained for the photoblock copolymerization of VAc. Thus, various kinds of two- or three-component block copolymers were prepared [157, 158].

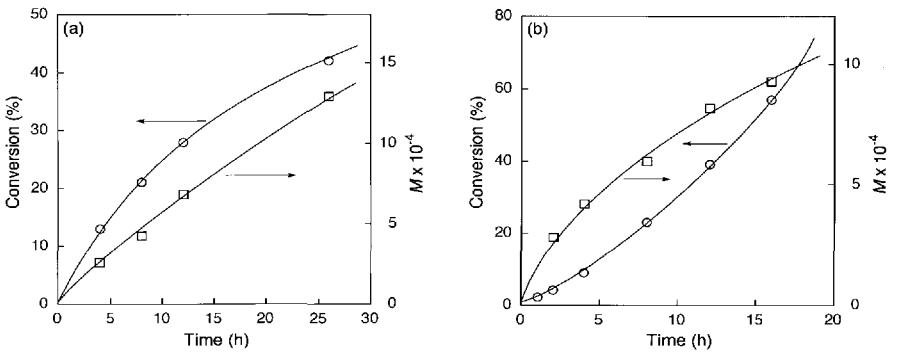


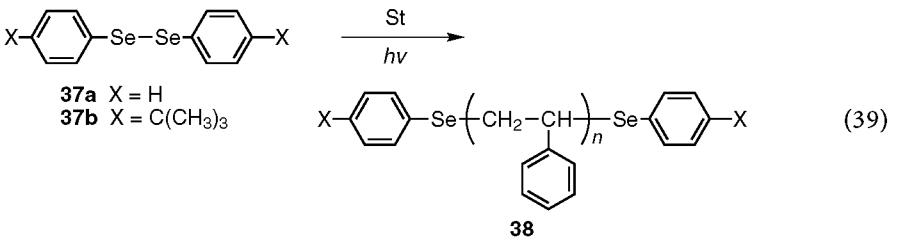
Fig. 2. Time-conversion and time-molecular weight relationships for photopolymerization of St in bulk (**a**) and MMA in benzene (**b**) with **13** at 30 °C. (a) [**13**] = 7.7 x 10^{-3} mol/L, (**b**) [MMA] = 4.7 mol/L, [**13**] = 4.6 x 10^{-3} mol/L.

Table 2. Radical Polymerization of St with Photoiniferters in Benzene at 30 °C [156]

Iniferter (mmol/L)	[St] (mol/L)	Time (h)	$M \times 10^{-4}$	N_{DC}[a]
13 (7.7)	7.7	8	2.1	1.7
		12	3.1	1.9
		24	5.7	2.0
7(7.8)	6.9	3	2.1	0.9
		6	3.2	0.9
		9	4.3	1.0
		12	5.5	1.1
		15	6.3	1.0
8(3.8)	6.9	3	3.8	1.8
		6	6.3	1.7
		9	9.5	1.9
		12	12.2	2.0
		15	15.4	2.0

[a] Number of DC groups per one polymer chain, determined by UV absorption.

Recently, Kondo and coworkers reported on the polymerization of St with diphenyl diselenides (**37**) as the photoiniferters (Eq. 39) [162]. In the photopolymerization of St in the presence of **37a** and **37b**, the polymer yield and the molecular weight of the polymers increased with reaction time. The chain-end structure of the resulting polymer **38** was characterized. Polymer **38** underwent the reductive elimination of terminal seleno groups by reaction with tri-*n*-butyltin hydride in the presence of AIBN (Eq. 40). It also afforded the poly(St) with double bonds at both chain ends when it was treated with hydrogen peroxide (Eq. 41). They also reported the polymerization of St with diphenyl ditelluride to afford well-controlled molecular weight and its distribution [163].



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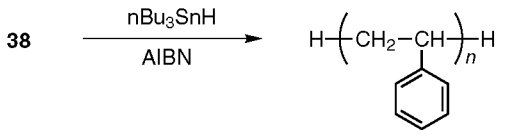
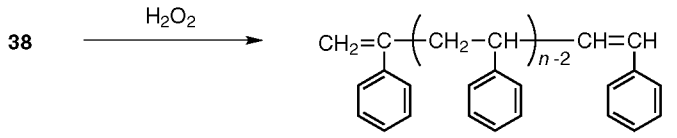
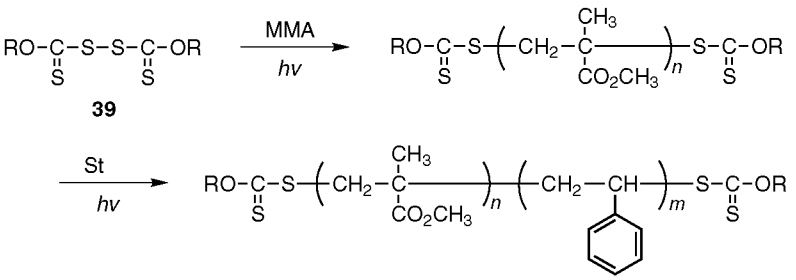


(40)



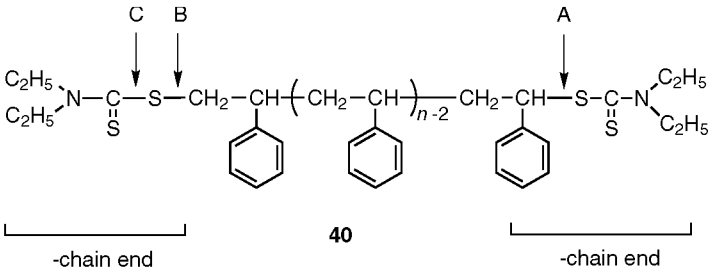
(41)

It has also been reported that xanthogen disulfide (**39**) can act as a photoiniferter of the polymerizations of St and MMA (Eq. 42), being very similar to the polymerization with **13** [164, 165]:



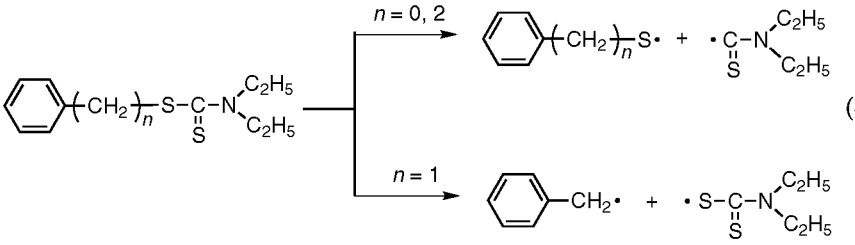
(42)

When **13** is used as a photoiniferter for the living radical polymerization, the thermal DC groups of both chain ends of **15** are not identical, i.e., the DC group bonds to the head and tail positions of the terminal St monomer units of the polymer, as shown in the structure of poly(St) **40**.



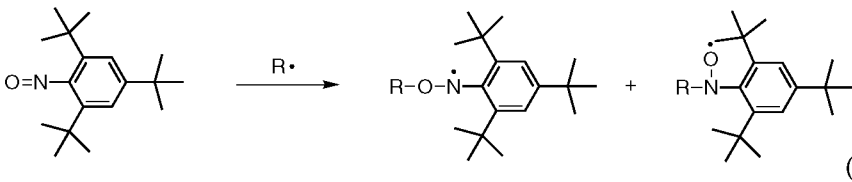
In the ω-end of the chain, the dissociation always occurs at the bond which is indicated by the arrow A. The dissociation of this C-S bond at the A position gives a more-reactive carbon-centered radical and a less-reactive polymer thiyl radical, which leads to the termination of the active chain ends. In the case of the α-chain end, however, there is a possibility that the bond at the C position dissociates to produce a diethylaminothiocarbonyl radical and a thiyl radical in addition to the preferable bond scission at B. Such dissociation at C may not induce living radical polymerization [76].

Okawara et al. reported the photodissociation of several carbamate derivatives on the basis of the product analysis [166]. They demonstrated that the cleavage bonds were different corresponding to the number of the methylene groups in Eq. (43). When n was unity, the reactive benzyl radical and less-reactive thiyl radical were produced.



(43)

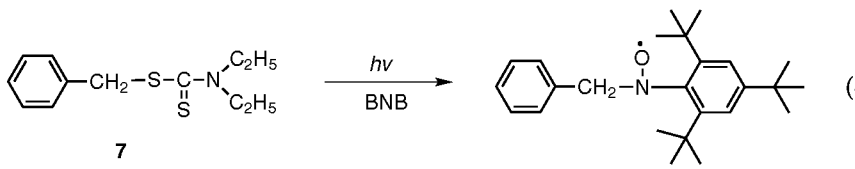
We have confirmed the dissociation manner of these compounds by means of the spin-trapping technique [167]. The radicals produced from 7 and 2-phenylethyl *N,N*-diethyldithiocarbamate (**41**) were trapped with 2,4,6-tri-*tert*-butylnitrosobenzene (BNB) as a spin-trapping agent (Eq. 44) [168]:



BNB

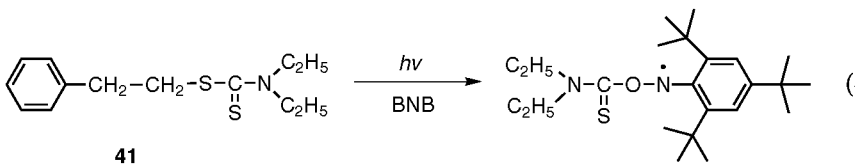
(44)

The reaction products from 7 and **41** were as follows:



7

(45)



41

(46)

These results indicate that the dissociation of **41** occurred at the C-S linkage to yield a phenylethyl thiyl radical and a diethyldithiocarbonyl radical, while 7 gave benzyl and DC radicals.

4.2.2
Iniferters as Polymer Chain-End Model

Otsu and Kuriyama designed photoiniferters which yield a highly reactive carbon radical and less reactive thiyl radical by photodissociation [169]. The former radical participates in propagation, and the latter acts only as a terminator. Bifunctional photoiniferters **8** as well as monofunctional **7** were prepared (see Eqs. 10 and 11 for the structures of **7** and **8**). These photoiniferters dissociate only at the easily dissociable benzylic C-S bond to give a benzylic radical similar to the propagating poly(St) radical and the less reactive DC radical.

The time-conversion and time-molecular weight relationships in the photopolymerization of St with **7** and **8** are shown in Fig. 3, in which the concentration of the DC group as an iniferter site in these iniferters was identical, i.e., [**7**]/2–[**8**].

Both time-conversion curves for the polymerization with **7** and **8** are superimposed on each other, indicating that the C-S bonds in **8** and the resulting poly(St) (**43**) may dissociate into benzylic and thiyl radicals with the same probability as those in **7** and **42**. This supports the thesis that the polymerization with these iniferters proceeds via a living radical mechanism, and that **42** and **43** serve as mono- and difunctional polymeric photoiniferters, respectively. The yield and molecular weight of the polymers increased as a function of the polymerization time, i.e., conversion. The slope of the curves of the molecular weight relationship for **8** was twice as large as that with **7**, i.e., the molecular weight of the polymer obtained with **8** was twice as high as that with **7**. Similar results were also found for the radical polymerization of MMA and another methacrylate with **7** and **8** [169, 170].

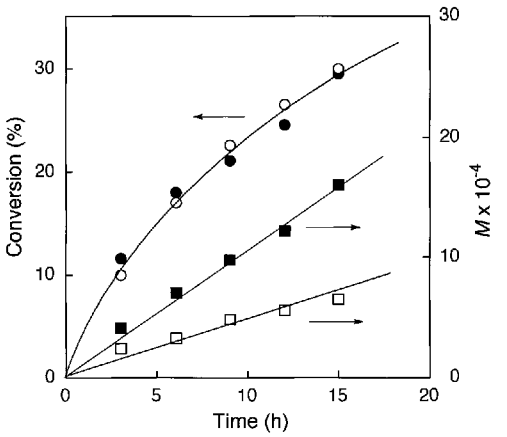
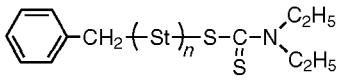


Fig. 3. Time-conversion and time-molecular weight relationships for photopolymerization of St with **7** and **8** in benzene at 30 °C. [St] = 6.9 mol/L, [**7**] = 7.8 x 10^{-3} mol/L, [**8**] = 3.8 x 10^{-3} mol/L. (○, □) **7**, (,) **8**.



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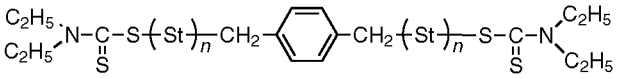


43

The changes in the molecular weight, molecular weight distribution, and the number of the DC end group of the poly(St) were determined as a function of the reaction time in the polymerization of St with **7** and **8**. The results are shown in Table 2 and Fig. 4 [156]. It is noted from Fig. 4 that the molecular weight distribution of the polymers increased with the reaction time (conversion), unlike living anionic polymerizations that provide polymers with a narrow molecular weight distribution close to unity. The number of the end group per one polymer chain are almost constant, i.e., 1 for **7** and 2 for **8**, independent of the polymerization time (Table 2). It strongly supports that these polymerizations are performed according to Eq. (18), and the polymers with the DC end groups are always reproduced.

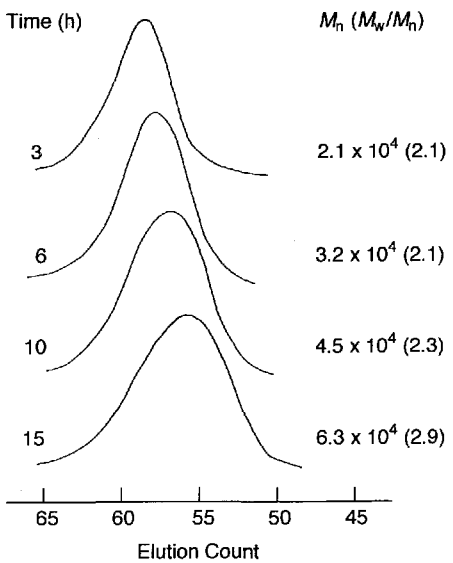
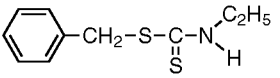


Fig. 4. GPC traces of the polymers obtained from photopolymerization of St with **7** in bulk at 30 °C. [7] = 7.3 x 10^{-3} mol/L.

From the slope of the line observed in the conversion-molecular weight relationship, the living nature in the polymerization of St or MMA with **13**, **7**, and **8** as photoiniferters was fairly high. However, the efficiency of the block copolymer formation was about 70–90 %, as described below, indicating that some undesirable side reaction leading to deactivation of the iniferter site as a dormant propagating radical species might occur during polymerization and disturb the ideal living radical polymerization, e.g., ordinary bimolecular termination, initiation by the DC radicals, and photodissociation of the bonds other than the specified bond leading to living radical polymerization, etc.

Benzyl *N*-ethyldithiocarbamate (**44**) and *p*-xylylene bis(*N*-ethyldithiocarbamate) (**45**) were also prepared as mono- and difunctional photoiniferters, respectively [171, 172], consisting of a structure similar to **7** and **8**. The polymerization of St with **44** under ultrasonic irradiation was also reported [173].



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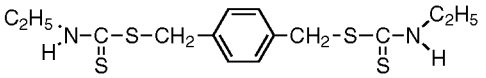
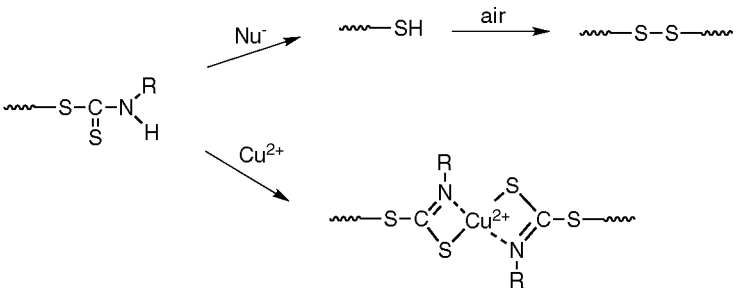


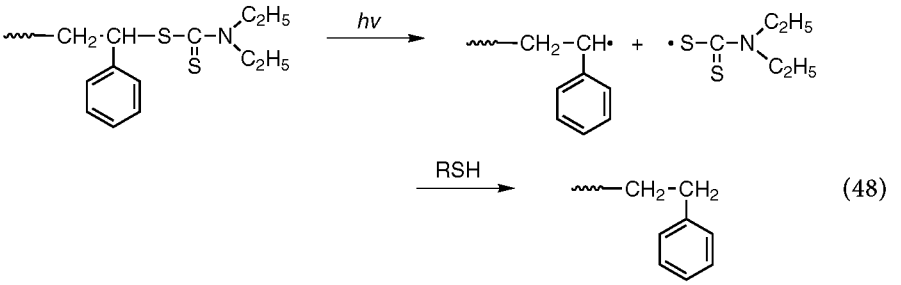
45

These iniferter sites containing an N-H group can be easily transformed into the corresponding thiol which leads to disulfide by oxidative coupling and can form chelation with metal ions (Eq. 47) [171, 172]. Poly(St) prepared for polymerization with **44** and **45** was applied to the chain-extension reaction by the S-S bond or chelation bond formations.



(47)

The polymers thus obtained with iniferters such as **7** having a photosensitive labile bond in their chain end are unfavorable for practical use and storage when the polymer is exposured under UV light. Therefore, stabilization of the chain end of the polymers, i. e., the removal of the DC group, was attempted [174]. The transformation of the ω-chain end structure by chain transfer was the most effective. When the polymer chain end was dissociated into the propagating radical and DC radical under UV irradiation, a thiol compound as a chain transfer agent made the polymer chain end inactive (Eq. 48). According to this procedure, the DC group could be detached without any change in the molecular weight of the polymer.

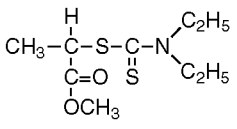


(48)

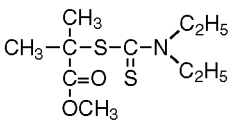
The polymerizations of some vinyl monomers other than St and MMA have also been characterized. In the polymerization of VAc in the presence of 7 as a photoiniferter, the molecular weight increased with the conversion, but it did not pass through the origin, i.e., with an intercept. In the case of the polymerization of acrylates, the molecular weight of the polymer obtained gradually decreased with the conversion [175]. Similar polymerization behaviors of the polymerization of acrylates were also reported by Lambrinos et al. [176]. They pointed out that the polymerization of BA showed some features of a living system, e.g., an increase in the molecular weight of the polymer and the preparation of block copolymers, suggesting a reversible termination between the resulting chains and the DC radicals. However, some evidence has also been found for the side reactions, such as the formation of **13**, a decrease in the functionality of the polymers. These results indicated that 7 does not act as an effective iniferter for the polymerization of MA and VAc, in contrast to the polymerizations of St and MMA.

These are two possibilities for the polymerization of MA deviated from the ideal living radical polymerization: (i) the chain end of poly(MA) formed primary radical termination with a DC radical does not dissociate or dissociates at an unfavorable position like **41**; (ii) bimolecular termination leading to the deactivation of the iniferter sites occurs preferentially to the primary radical termination with the DC radical which reproduces the iniferter site.

The dissociation of model compounds for ω-chain ends of polymers obtained using iniferters with the DC group was examined by the spin-trapping technique, similar to the dissociation of 7 and 8 previously mentioned [174, 175]. From the results of the trapping experiments, it was concluded that **46**, **47**, and **48** as model compounds for poly(MA), poly(MMA), and poly(VAc), respectively, dissociated at the appropriate position to produce a reactive carbon-centered radical and a stable DC radical. In fact, these compounds were found to induce the living radical polymerization of St when they were used as photoiniferters.



46



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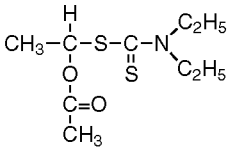


48

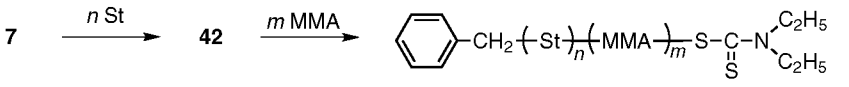
The former possibility previously described could be refuted by the spin-trapping experiments and the living radical polymerization of St with **46**. Therefore, **13** was added to the polymerization system to conserve the active site of the iniferter. It was expected to reproduce the iniferter site due to the formation of DC radicals which can function as primary radical terminators and/or the effective chain transfer ability of **13**. It was pointed out that the DC radical generated from **13** had high selectivity for monomers, i.e., **13** acted as an initiator for the polymerization of St, but did not as an initiator for the polymerization of MA, VAc, and AN [72, 175, 177].

The polymerization of MA with **7** was carried out in the presence of **13**, i.e., **7** and **13** were used as two-component iniferters [175]. When an identical amount of **13** to **7** was added to the system, the polymerization proceeded according to a mechanism close to the ideal living radical polymerization mechanism. Similar results were also obtained for the polymerization of VAc. These results indicate that the chain end of the polymer was formed by the competition of primary radical termination and/or chain transfer to bimolecular termination, and that it could be controlled by the addition of **13**.

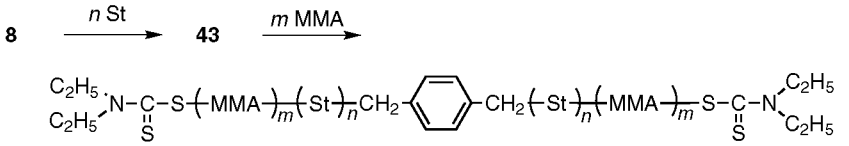
5
Design of Block, Star, and Graft Polymer Syntheses with Dithiocarbamyl Compounds as Iniferters

5.1
AB- and ABA-Type Block Copolymers

As previously described, the polymers obtained by **7** and **8** further serve as mono- and difunctional photoiniferters, respectively. If the poly(St)s **42** and **43** are used for the polymerization of MMA as a second monomer, AB- and ABA-type block copolymers, respectively, would be synthesized, as shown in Eqs. (49) and (50):



(49)



(50)

Table 3. Block copolymerization with polymeric photoiniferters[a] [178]

Polymeric Photoiniferter	Monomer (M_2)	Time (h)	Fraction Extracted (%)		
			Unreacted Iniferter	M_2 Homopolymer	Block Copolymer
7-poly(St)	MMA	7	7	15	78
8-poly(St)	MMA	7	2	9	89
8-poly(MMA)	St	10	14	23	62
8-[poly(St)-*co*-poly(MMA)]	VAc	8	5	13	82
8-[poly(St)-*co*-poly(MMA)]	AA	0.3	3	9	88
8-[poly(St)-*co*-poly(IB)]	MMA	3	15	19	66
8-[poly(St)-*co*-poly(VAc)]	MMA	3	14	3	83
8-[poly(St)-*co*-poly(VCl)]	MMA	3	9	12	79
7-poly(St)	MAn/IBVE	6	0	42	58
8-poly(St)	MAn/IBVE	6	0	31	69
7-[poly(St)-*co*-poly(DiPF)]	MAn/IBVE	4	8	29	63
8-[poly(St)-*co*-poly(DiPF)]	MAn/IBVE	4	6	24	70

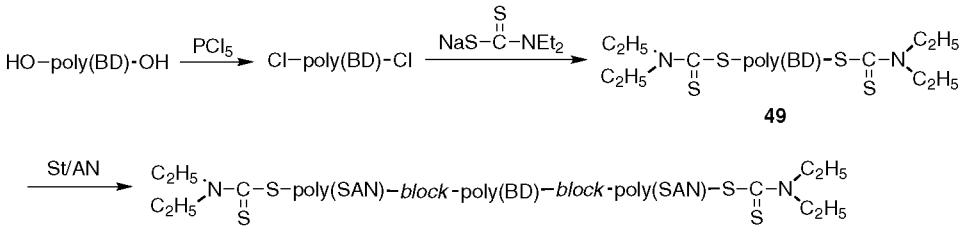
[a] St: styrene, MMA: methyl methacrylate, IB: isobutene, VAc: vinyl acetate, VCl: vinyl chloride, DiPF: diisopropyl fumarate, AA: acrylic acid, MAn: maleic anhydride, IBVE: isobutyl vinyl ether.

The results of the block copolymerization of St , MMA, AA, and VAc with the polymers obtained by **7** and **8** are shown in Table 3. The yields of the block copolymers with **42** and **43** were as high as 70–90%. These block copolymer syntheses are advantageous for the synthesis of the polymer consisting of many kinds of vinyl monomer units, especially polar and functional monomers.

A similar technique was applied to the synthesis of AB and ABA block copolymers containing random and alternating copolymer sequences [178–180]. For example poly(St-*random*-MMA)-*block*-poly(VAc), poly(VAc-*block*-poly(St-*random*-MMA)-*block*-poly(VAc), poly(St)-*block*-poly(DiPF-*alt*-IBVE), poly(IBVE-*alt*-MAn)-*block*-poly(St)-*block*-poly(IBVE-*alt*-MAn), poly(St)-*block*-poly(EA)-*random*-AA),and poly(St)-*block*-poly(EA-*random*-AA-*random*-MMA) were synthesized [178].

De Simone et al. synthesized poly(fluoroalkyl acrylate)-based block copolymers for use as lipophilic/CO_2-philic surfactants for carbon dioxide applications [181]. The particle diameter and distribution of sizes during dispersion polymerization in supercritical carbon dioxide were shown to be dependent on the nature of the stabilizing block copolymer [182].

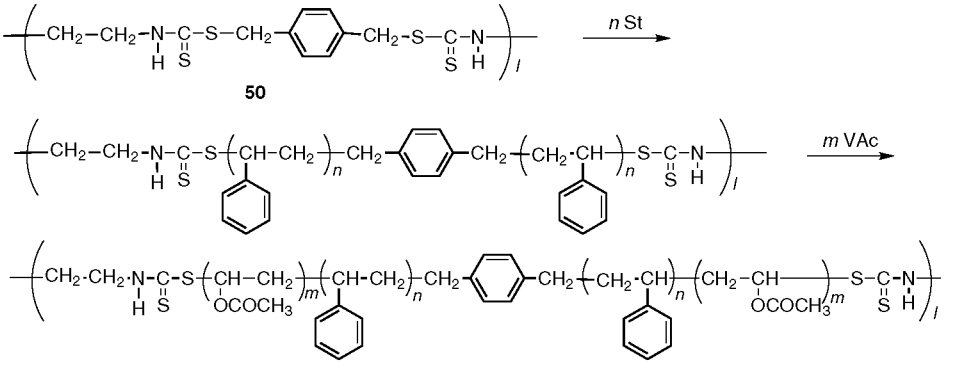
Recently, bifunctional polymeric iniferter **49** was synthesized and applied to the preparation of the triblock copolymer, poly(SAN)-*block*-poly(BD)-*block*-poly(SAN) [183]:



(51)

Synthesis and application using polymeric photoiniferters based on poly(DMS) and polyurethanes are found in a review by Kumar et al. [184].

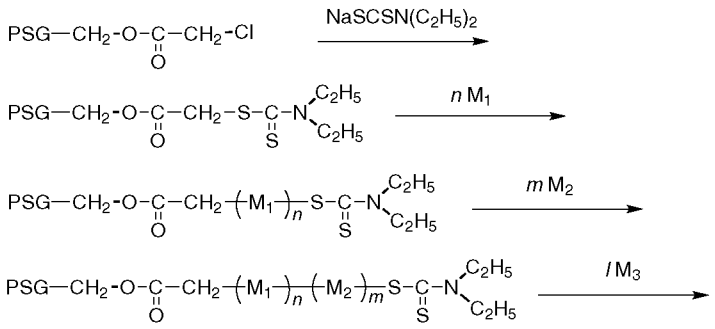
When the polyfunctional iniferter **50** which has several DC units in the main chain was used, multiblock copolymers of St and VAc were easily prepared, as shown in the following equation [185]:

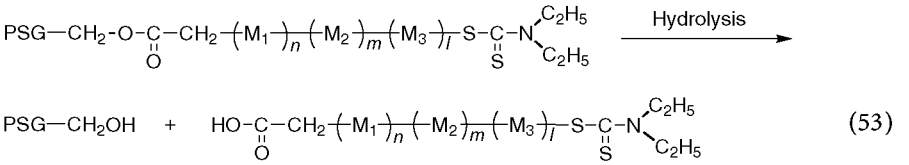


(52)

5.2 Solid-Phase Block Copolymer Synthesis

The synthesis of some multiblock copolymers was attempted by successive polymerization using this iniferter technique. However, pure tri- or tetrablock copolymers free from homopolymers were not isolated by solvent extraction because no suitable solvent was found for the separation. In 1963, Merrifield reported a brilliant solid-phase peptide synthesis using a reagent attached to the polymer support. If a similar idea can be applied to the iniferter technique, pure block copolymer could be synthesized by radical polymerization. The DC group attached to a polystyrene gel (PSG) through a hydrolyzable ester spacer was prepared and used as a PSG photoiniferter (Eq. 53) [186]:





(53)

After photopolymerization of a certain monomer for a given time, the polymerization mixture was poured into excess precipitant to isolate the polymer, which was then extracted with a solvent to separate the polymer grafted onto the PSG from the homopolymer.

For example, the grafted poly(St) onto PSG was observed to act as a photoiniferter for the radical polymerization of MMA. After the separation of the homopolymer of MMA from the resulting graft-block copolymer attached to PSG by solvent extraction, a block copolymer of poly(St) with poly(MMA) was isolated by hydrolysis and the subsequent solvent extraction. The yield and molecular weight increased with the reaction time. From GPC measurement, however, the block copolymer thus obtained was revealed to contain 10–15% homopoly(St), indicating that a chain end was deactivated during the polymerization of St as a result of an increased bimolecular termination between the chain ends of the polymers fixed on the gel. To avoid homopoly(St) formation, **13** was added to the photopolymerization of St. When the polymer grafted onto PSG thus obtained was used as a PSG photoiniferter for the polymerization of MMA, followed by extraction and hydrolysis of the resulting graft-block copolymer, a block copolymer of poly(St) with poly(MMA) which contained only a trace of homopoly(St) was iso-

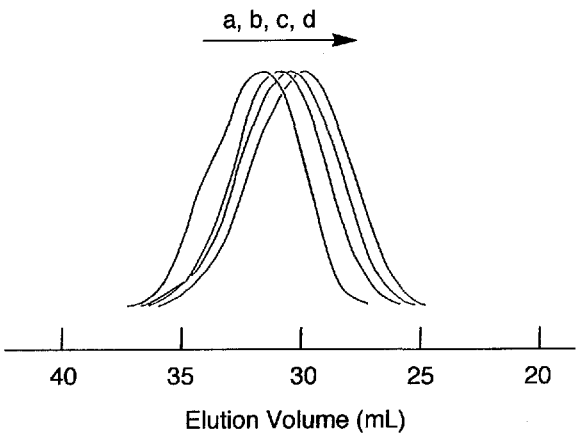


Fig. 5. GPC elution curve of bock copolymers. (a) poly(St), (b) poly(St)-*block*- poly(MMA), (c) poly(St)-*block*-poly(MMA)-*block*-poly(EMA), and (d) poly(St)-*block*-poly(MMA)-*block*-poly(EMA)-*block*-poly(MOSt).

lated. The graft-block copolymer consisting of an St-MMA block attached to PSG obtained by the polymerization of MMA in the presence of the graft polymer of St to PSG and **13** was confirmed to induce further photopolymerization, leading to pure poly(St)-*block*-poly(MMA)-*block*-poly(St) triblock copolymer. In a similar way, poly(St)-*block*-poly(MMA)-*block*-poly(ClSt) as ABC-type triblock copolymer, poly(St)-*block*-poly(MMA)-*block*-poly(St)-*block*-poly(MMA) and poly(St)-*block*-poly(MMA)-*block*-poly(EMA)-*block*-poly(MOSt) as ABAB- and ABCD-type tetrablock copolymers, respectively, were also prepared. These block copolymer syntheses were confirmed by the GPC and NMR measurements of the polymers which were isolated at each step, as shown in Figs. 5 and 6 [24].

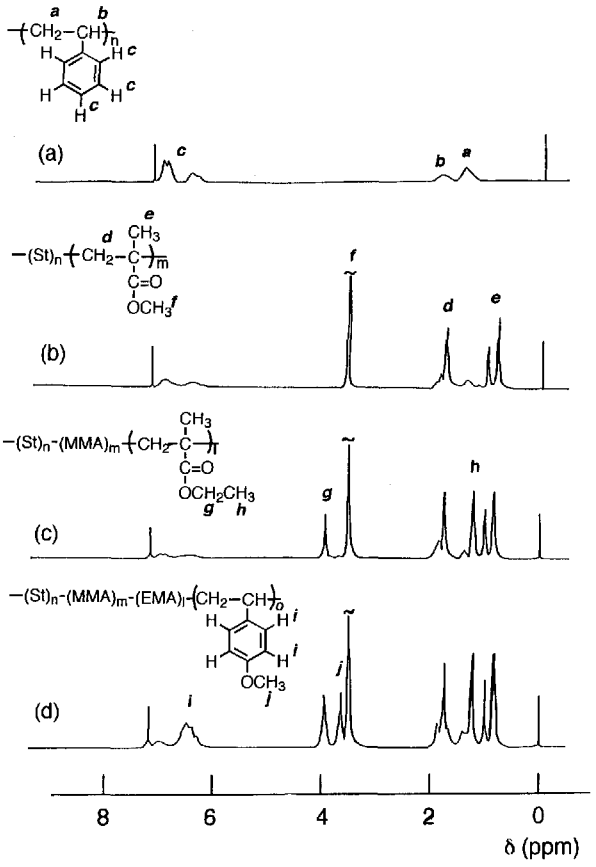
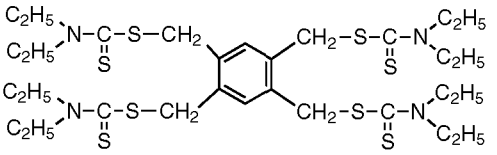


Fig. 6. ^{1}H NMR spectra of block copolymers. (a) poly(St), (b) poly(St)-*block*-poly(MMA), (c) poly(St)-*block*-poly(MMA)-*block*-poly(EMA), and (d) poly(St)-*block*-poly(MMA)-*block*-poly (EMA)-*block*-poly(MOSt).

5.3
Synthesis of Star and Graft Polymers

5.3.1
Star Polymers

1,2,4,5-Tetrakis(*N,N*-diethyldithiocarbamyl)benzene (**51**) was prepared as a tetrafunctional photoiniferter [187]:



51

When the polymerization of St was carried out with **51** under conditions identical to those in Fig. 3, i.e., [**7**]/4=[**8**]/2=**51**=2×10^{-3} mol/l, the formation of benzene-insoluble polymers was observed from the initial stage of the polymerization. Although **7** and **8** induced living radical mono and diradical polymerization similar to that previously mentioned, benzene-insoluble polymers were formed in the polymerization with **51**, and the molecular weight of the soluble polymers separated decreased with the reaction time. This suggests that a part of the propagating polymer radicals underwent ordinary bimolecular termination by recombination, leading to the formation of the cross-linked polymer, which was prevented by the addition of **13**.

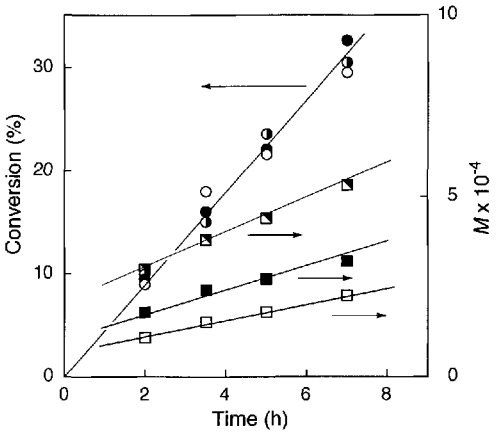


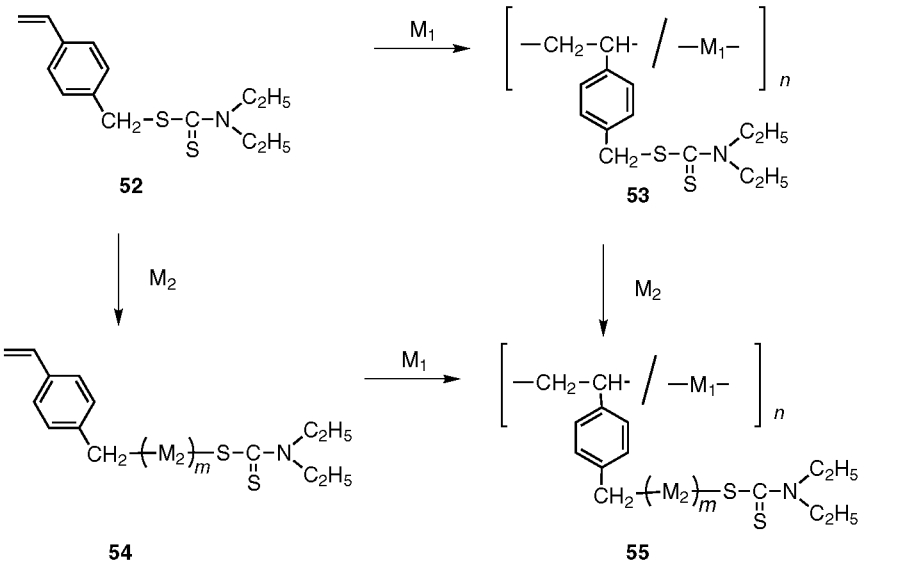
Fig. 7. Time-conversion and time-molecular weight relationships for photopolymerization of MMA with **7**, **8**, and **51** in benzene at 30 °C. [MMA] = 7.5 mol/L, [**7**] = 5.4 x 10^{-4} mol/L, [**8**] = 2.6 x 10^{-4} mol/L, [**51**] = 1.3 x 10^{-4} mol/L. (○, □) **7**, (,) **8**, (◑, ◥) **51**.

The results of similar polymerization using MMA are shown in Fig. 7. The plots of the polymer yield with the reaction time were on the identical straight line, and no benzene-insoluble polymers were produced, being different from that for St. Moreover, the results of Fig. 7 suggest that the four C-S bonds in the photoiniferter sites show identical reactivity during the polymerization of MMA. Although the molecular weight of the poly(MMA) obtained increases with time, the relative ratio of increasing molecular weight was 1.0, 1.7, and 2.5 for **7**, **8**, and **51**, respectively. If they act as ideal photoiniferters, the ratio must be 1, 2, and 4, indicating that the polymerization of MMA with **7**, **8**, and **51** is beyond the ideal living mono-, di-, and tetra-radical mechanism, due to unfavorable side reactions, such as self-termination. It might also be attributed to the uncertainty of the molecular weights determined by viscometrical and GPC measurements, because of the alteration of the polymer shape from linear to star.

These poly(MMA) obtained could induce the photopolymerization of St to give a star block copolymer, but gelation was partly observed, similar to the polymerization of St with **51**. The addition of **13** in the photopolymerization of MA with **51** was effective in preventing gelation [175].

5.3.2
Graft Copolymers

If the DC photoiniferter having a polymerizable double bond, i.e., a monomer iniferter, is successively used as both monomer and iniferter, macromonomers and graft copolymers would be obtained according to Eq. (54) [188]:



(54)

For this purpose, 4-vinylbenzyl *N,N*-diethyldithiocarbamate (**52**) was prepared and used for the synthesis and design of graft and cross-linked polymers. In the absence of light, **52** easily polymerized in the presence of an azo initiator. The resulting homopoly(**52**) or its copolymers with St (**53**) were found to act as polyfunctional photoiniferters of living radical polymerization leading to a graft copolymer consisting of benzene-soluble and -insoluble fractions, in which the amount of the latter increased when the **52** units increased in the copolymer. The polymerization of St from **53** yielded only an insoluble copolymer, but the graft copolymerizations of MMA and MA gave both soluble and insoluble fractions. When **13** was added in the polymerization system of MA, soluble graft copolymer was obtained in a high yield [175].

The photopolymerization of St with catalytic amount of **52** as the photoiniferter gave a benzene-soluble polymer that contains a styryl double bond and a DC group at the polymer chain ends. When this macromonomer-iniferter **54** was copolymerized with a second monomer in the presence of an azo initiator, the formation of a high molecular weight graft copolymer was confirmed by GPC data. The monomer iniferter **52** was also used for the preparation of photoresist polymers [189].

Nakayama and Matsuda [190] recently succeeded in the design of the surface macromolecular architecture with regional dimensional precision, control of the thickness of a graft layer, and blocks of graft chains using photograft copolymerization by the UV irradiation of a DC group immobilized polymer surface in the presence of vinyl monomers. X-ray photoelectron spectroscopy and water contact angle confirmed that the graft copolymerization proceeded only during photoirradiation and at photoirradiated portions. From atomic force microscopic observations, it was found that the thickness of the graft layers increased linearly with irradiation time. The use of a projection mask during irradiation and the sequential monomer charges provided the polymer surfaces with regionally and dimensionally controlled polymers such as di- and triblock graft copolymerized surfaces. A thickness-gradient graft surface was also obtained using a gradient filter. This method will be useful for functional surface design for artificial organs, micromachines, and microbiosensors.

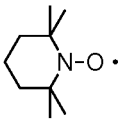
Poly(VCl)-based polymeric iniferters bearing the DC, xanthates, and mercaptobenzothiazole moieties into the side chain provide graft copolymers [191–193]. Graft copolymerizations from poly(St) and poly(DMS) modified with the DC moiety were also reported [194, 195]. The graft copolymerization of methoxy polyethylene glycol methacrylate and *N,N*-dimethylaminoethyl methacrylate was carried out using poly(ethylene-*co*-VAc)-*graft*-poly(VCl) modified with the DC groups, followed by quaternilization to form an ionic complex with heparin [196–201]. These copolymers have been applied to biomedical uses, including polymeric materials for catheters.

6
Recent Developments in Living Radical Polymerization

6.1
Living Radical Polymerization of Styrene with TEMPO

6.1.1
Synthesis of Poly(St) with a Narrow Molecular Weight Distribution

In 1993, Georges and coworkers [23, 202, 203] first succeeded in the synthesis of poly(St) with a narrow molecular weight distribution through the free-radical polymerization process of St. The polymerization was carried out in the presence of BPO and 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO):



TEMPO

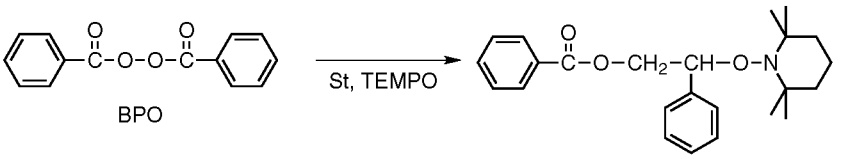
The reaction mixture was heated at 95 °C for 3.5 h, and then the temperature was raised to 123 °C to give a polymer with the M_w/M_n value of 1.2–1.3. As the polymer yield increased, the M_n value also increased keeping the low M_w/M_n value (Table 4). For example, the 69-h polymerization provided poly(St) with an M_n of 7.8×10^3 and M_w/M_n of 1.27 in 90% yield. The excess TEMPO is necessary for the control of M_w/M_n. This living radical polymerization was also applied to the suspension copolymerization of St with butadiene, giving the random copolymer with a narrow molecular weight distribution (M_w/M_n=1.36). The living radical polymerization in an emulsion system has recently been investigated [204].

Hawker isolated the adduct **56** in 42% yield in the reaction of St with BPO and TEMPO at 80 °C, as shown in Eq. (55) [205]:

Table 4. Living Radical Polymerization of St with TEMPO and BPO[a] [202]

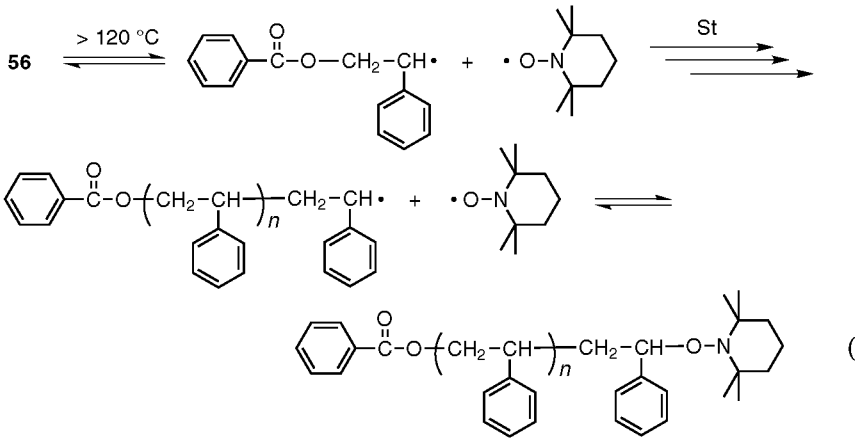
TEMPO/BPO	Time (h)	Yield (%)	M_n x 10^{-3}	M_w/M_n
1.2	21	20	1.7	1.28
1.2	29	51	3.2	1.27
1.2	45	76	6.8	1.21
1.2	69	90	7.8	1.27
0.5	–	86	45.6	1.57
1.5	–	74	33.1	1.24
3.0	–	71	18.2	1.19

[a] Polymerization temperature: 95 °C for 3.5 h and then 123 °C.



(55)

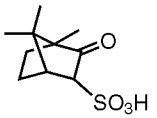
The polymerization of St with **56** as the initiator is considered to procced via a reaction mechanism in Eq. (56), being identical to the models in Eqs. (18) and (20). The structure of both chain ends of the resulting polymer was confirmed by NMR using the deuterated St as the monomer. The polymerization with BPO and TEMPO without isolation of the adduct would also proceed via a similar path. In the absence of BPO, it has been reported that the radicals produced by spontaneous initiation according to the Mayo mechanism react with TEMPO to yield the adducts, and then they initiate polymerization [206].



(56)

The living radical polymerization process is also valid for the polymerization of water-soluble monomers. The polymerization of sodium styrenesulfonate in aqueous ethylene glycol (80%) in the presence of TEMPO using potassium persulfate/sodium bisulfite as the initiator at 125 °C gave a water-soluble polymer with well-controlled molecular weight and its distribution [207].

This living radical poylmerization of St proceeds at a slow rate even at a high temperature such as 125 °C, being a disadvantage to an industrial application. It has been reported that the addition of camphorsulfonic acid (**57**) and 2-fluoro-1-methylpyridinium *p*-toluenesulfonate (**58**) enhanced the polymerization rate [208–211]. For example, a polymer with an M_n of 2.16×10^4 and M_w/M_n of 1.26 was produced in 76% yield during the 5.5-h polymerization in the presence of **57** at 2×10^{-2} mol/L, whereas the yield, M_n, and M_w/M_n were 24%, 8.8×10^3, and 1.13, respectively, in the absence of **57**. Scaiano and coworkers [212] investigated the rate constant of the bond formation between the benzyl radical and TEMPO by laser flash photolysis and revealed that **57** decreased the rate of bond formation.



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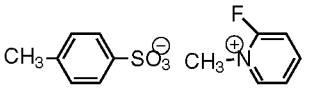
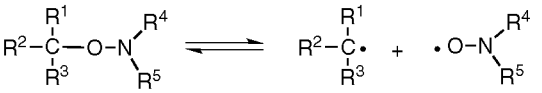


58

The synthesis of poly(St) with a narrow molecular weight distribution by Georges and colleagues has attracted great interest from many researchers of living radical polymerization in both the fundamental and applied fields. An impressive number of articles have been published since 1993 by a number of research groups, being classified into the analysis of the reaction mechanism and the architecture of polymer structures. They are reviewed in the following sections.

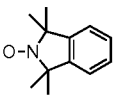
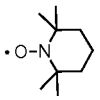
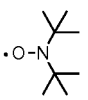
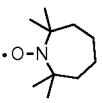
6.1.2
Reaction Mechanism of Living Radical Polymerization with TEMPO

An alkyl radical and a nitroxide radical exist in an equlibrium with the corresponding alkoxyamine as their coupling product (Eq. 57). Moad and Rizzardo [213] and Kazmaier et al. [214] independently estimated the effects of the structure of the alkyl group and the nitroxide on the dissociation energy of various alkoxyamines into the radicals by semiempirical molecular orbital calculations. The bond dissociation energies determined are summarized in Table 5:



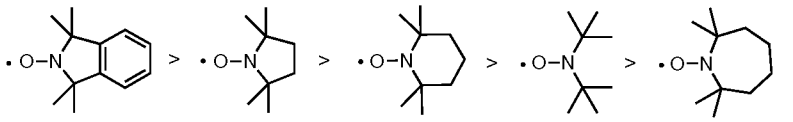
(57)

Table 5. Radical Dissociation Energy for Alkoxyamines (unit in kJ/mol)[a]

Alkyl Group Structure			Nitroxide Structure				
							
R^1	R^2	R^3					
CH_3	CH_3	CH_3	75.9	66.2	56.5	51.9	48.9
CH_3	CH_3	CN	–	61.7	–	–	–
CH_3	CH_3	Ph	–	80.5	–	–	–
CH_3	CH_3	H	105.6	101.0	98.2	92.3	86.7
CH_3	Ph	H	–(100)	96.9(71)	–(92)	–(71)	–
CH_3	H	H	134.0	130.1	130.5	127.7	124.8
H	H	H	165.8	161.9	162.5	159.6	156.6

[a] Results by Moad et al.[213]. The values in parentheses are the data by Kazmaier et al.[214].

It was clarified that the dissociation energy of the alkoxyamine decreased in the following order:

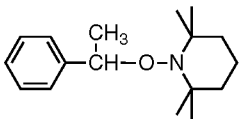


(58)

When the alkyl group is tertiary, the dissociation energies are small and dependent on the nitroxide structure, whereas the energy changes less-sensitively depending on the structure of the nitroxide when the alkyl group is methyl or primary alkyls. The nitroxides of the *sec*-alkyl groups have intermediate values. The alkyl groups importantly affect the dissociation behavior of the nitroxides.

It was reported that the enthalpies for the addition of the nitroxides to St are positive (approximately 30 kJ/mol), i.e., the addition reaction is unfavorable energetically, while the addition of the DC radical has a negative enthalpy, supporting the possibility of the initiation of St polymerization by the DC radical [214].

It was found that the adduct **59** also induces living radical polymerization similar to **56**, but the adduct **60** does not [215]. In the polymerization of St with **60**, the molecular weight did not increase with conversion, and a broad molecular weight distribution, i.e., M_w/M_n of 1.5–2.2 was observed. The half-life time was determined to be 5–10 min at 123 °C for **59**, while that of **60** is much longer (ca. 150 min). The dissociation properties of the alkoxiamines used determined the nature of the polymerization with **59** and **60**.



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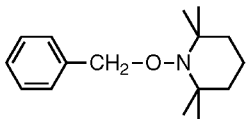


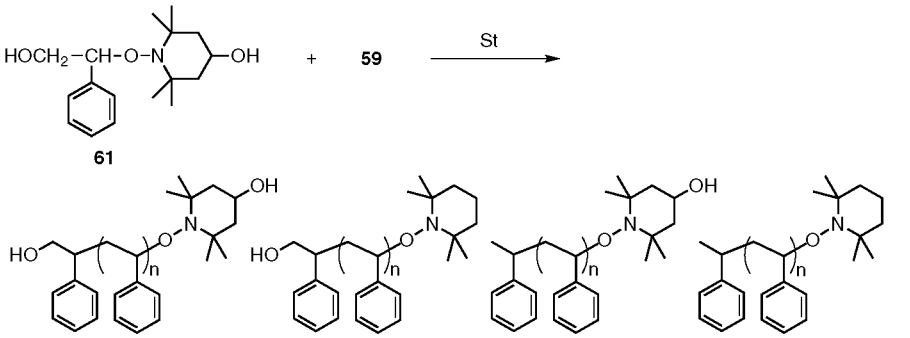
60

As is expected from these results, it is very difficult to control the polymerization of monomers other than St, e.g., that of MMA, because of the too small dissociation energy of the chain end of poly(MMA). In fact, the polymerization of MMA in the presence of TEMPO yielded the polymer with constant M_n irrespective of conversion, and the M_w/M_n values are similar to those of conventional polymerizations [216]. The disproportionation of the propagating radical and TEMPO would also make the living radical polymerization of MMA difficult. In contrast, the controlled polymerization of MA, whose propagating radical is a secondary carbon radical, has recently been reported [217]. Poly(MA) with a narrow molecular weight distribution and block copolymers were obtained.

For the design of the living radical systems in the polymerization of acrylates and methacrylates, arylazoxyl [218] and dialkyl borate [219] radicals are used and applied to the synthesis of the block copolymers, although the molecular weight distribution of the resulting polymers is broad in these polymerizations. The nitrogen radical as the stable radical was also examined for the polymerization of MMA and St [220]. Further investigation of the polymerization

system using various types of stable radicals is important for the expansion of the kind of monomers that can be used for living radical polymerization [221, 222].

The structure of the nitroxide moiety affects less importantly its dissociation ability, leading to difficulty in reaction control by the design of the nitroxyl groups. Because the propagation radical produced may react as the ordinary free radical species after the homolytic dissociation at the propagating chain end, the control of the propagation manner such as tacticity would not be expected in this system. The introduction of the chiral centers into the nitroxide moiety influenced the energetic property of the dissociation, but the tacticity of the polymers was the same as that of a conventional polymer [223]. This lessened sensitivity of the nitroxide structure in the living nature means that a variety of designs of the polymer end structure are possible. The free radical propagation was confirmed by cross-propagation experiments with **59** and **61** as the initiators for the living radical polymerization of St [224]. As expected, the polymerization provided the polymers with four kinds of chain-end structures as depicted in Eq. (59):



(59)

The polymerization kinetics have been intensively discussed for the living radical polymerization of St with the nitroxides, but some confusion on the interpretation and understanding of the reaction mechanism and the rate analysis were present [223, 225–229]. Recently, Fukuda et al. [230–232] provided a clear answer to the questions of kinetic analysis during the polymerization of St with the poly(St)-TEMPO adduct (M_n=2.5×10^3, M_w/M_n=1.13) at 125 °C. They determined the TEMPO concentration during the polymerization and estimated the equilibrium constant of the dissociation of the dormant chain end to the radicals. The adduct P-N is in equilibrium to the propagating radical P• and the nitroxyl radical N• (Eqs. 60 and 61), and their concentrations are represented by Eqs. (62) and (63) in the derivative form. With the steady-state equations with regard to P• and N•, Eqs. (64) and (65) are introduced, respectively:

$$\mathrm{P\bullet} + \mathrm{N\bullet} \underset{k_d}{\overset{k_c}{\rightleftharpoons}} \mathrm{P\text{–}N} \qquad (60)$$

$$K = k_d/k_c \tag{61}$$

$$d[P\bullet]/dt = R_i - k_t[P\bullet]^2 + k_d[P\text{-}N] - k_c[P\bullet][N\bullet] \tag{62}$$

$$d[N\bullet]/dt = k_d[P\text{-}N] - k_c[P\bullet][N\bullet] \tag{63}$$

$$[P\bullet] = (R_i/k_t)^{1/2} \tag{64}$$

$$[N\bullet] = K\,[P\text{-}N]/[P\bullet] \tag{65}$$

Here, R_i is the initiation rate, k_t is the rate constant for the bimolecular termination, and K is the equilibrium constant. From Eq. (64), the polymerization rate R_p is represented as

$$\begin{aligned} R_p\ (= -d[M]/dt\ ) &= k_p[P\bullet][M] \\ &= (k_p^2 R_i/k_t)^{1/2}[M] \end{aligned} \tag{66}$$

This equation is similar to that for the ordinary polymerization, indicating that R_p is independent of the concentration of P-N. In fact, the polymerization rate experimentally determined in the presence of P-N agreed with the rate of thermally initiated polymerization without any initiators. The production of the polymer induced a decrease in the k_t value because of the gel effects, resulting in an increase in the rate. The suppressed gel effects in the presence of TEMPO have also been reported [233]. Catala et al. interpreted the independence of the polymerization rate from the nitroxide concentration with the terms of the association of domant species. However, there is no experimental evidence for the association [229, 234, 235].

From the direct observation of the polymerization system by ESR spectroscopy, the concentration of N• was determined [231], whereas [P•] was calculated from the polymerization rate at each conversion because of the difficulty of the direct determination of low [P•] values. The [N•] value increased during the initial period of the poymerization and reached to $4\text{–}6\times10^{-5}$ mol/L. [P•] was estimated to be $1\text{–}2\times10^{-8}$ mol/L. The K value was estimated to be 2.1×10^{-11} with the experimentally determined values and Eq. (65), being constant during polymerization. If k_c is assumed to be $10^8\text{–}10^9$ L/mol s, then P-N dissociates one per 50–500 s, 0.6–6 molecules of St react, and then P• is combined with N• within 30–300 ms, resulting in the dormant species P-N.

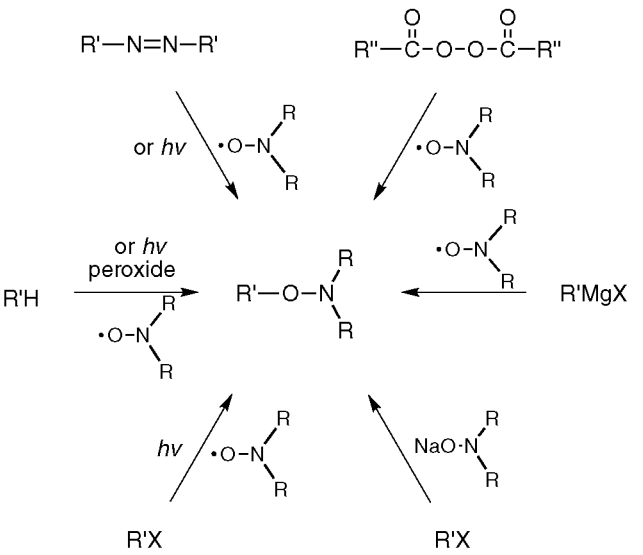
Thermal initiation and ordinary bimolecular termination also occur during polymerization in addition to initiation by the dissociation of the adduct or the active polymer chain-end dissociation and reversible temination (formation of the dormant species). Therefore, the degree of the control of the molecular weight and the molecular weight distribution is determined by the ratio of the polymer chains produced under control and uncontrol. If the contribution of the thermal initiation and bimolecular termination is very small, the molecular weight distribution is close to the Poisson distribution, i.e., $M_w/M_n = 1 + 1/P_n$, where P_n is the degree of polymerization. It was shown that when the number of

the polymer chains produced from thermal initiation is less than 15% of the controlled polymer chains, M_w/M_n is controlled to be less than 1.1.

Matyjaszewki et al. [229, 236] pointed out the importance of the bimolecular exchange reaction (Eq. 19) to control the molecular weight and its distribution. Simulation revealed a decrease in the M_w/M_n values during polymerization, but the contribution in the actual polymerization is still ambiguous [237–240]. Reports have also addressed the importance of the decomposition of the alkoxyamine such as the disproportionation of the propagating radical and the nitroxide for the control of the polymerization [229, 236, 241].

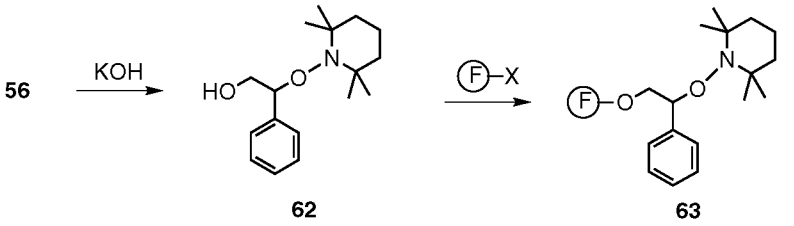
6.1.3
Architecture of the Polymer Structures

A variety of alkoxyamines are synthesized via various reaction routes, as summarized in Eq. (67), and are used for living radical polymerization:

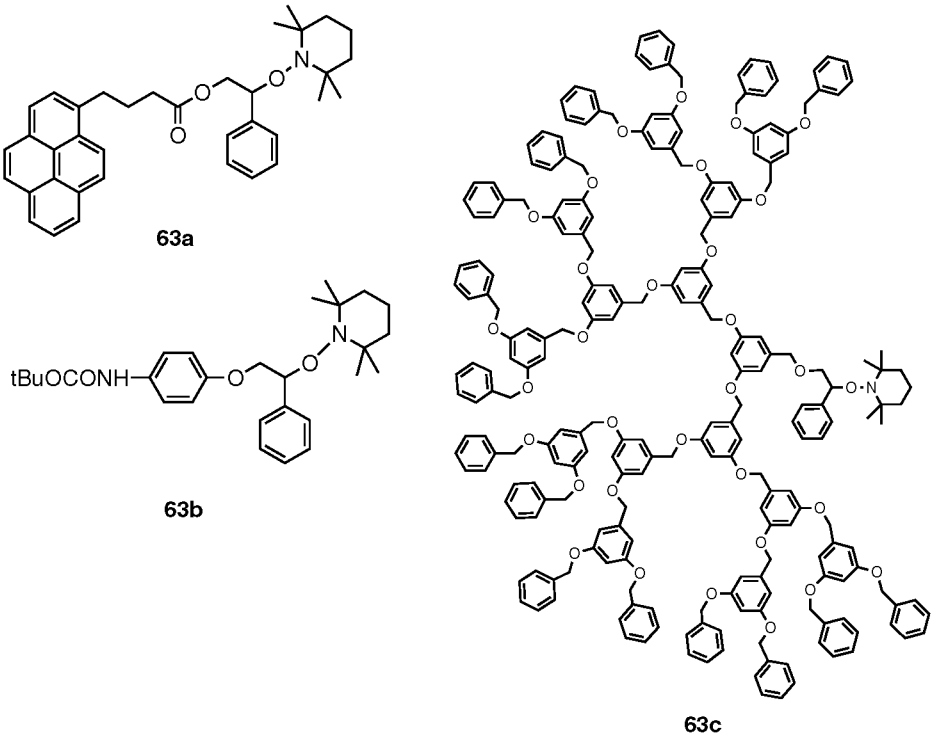


(67)

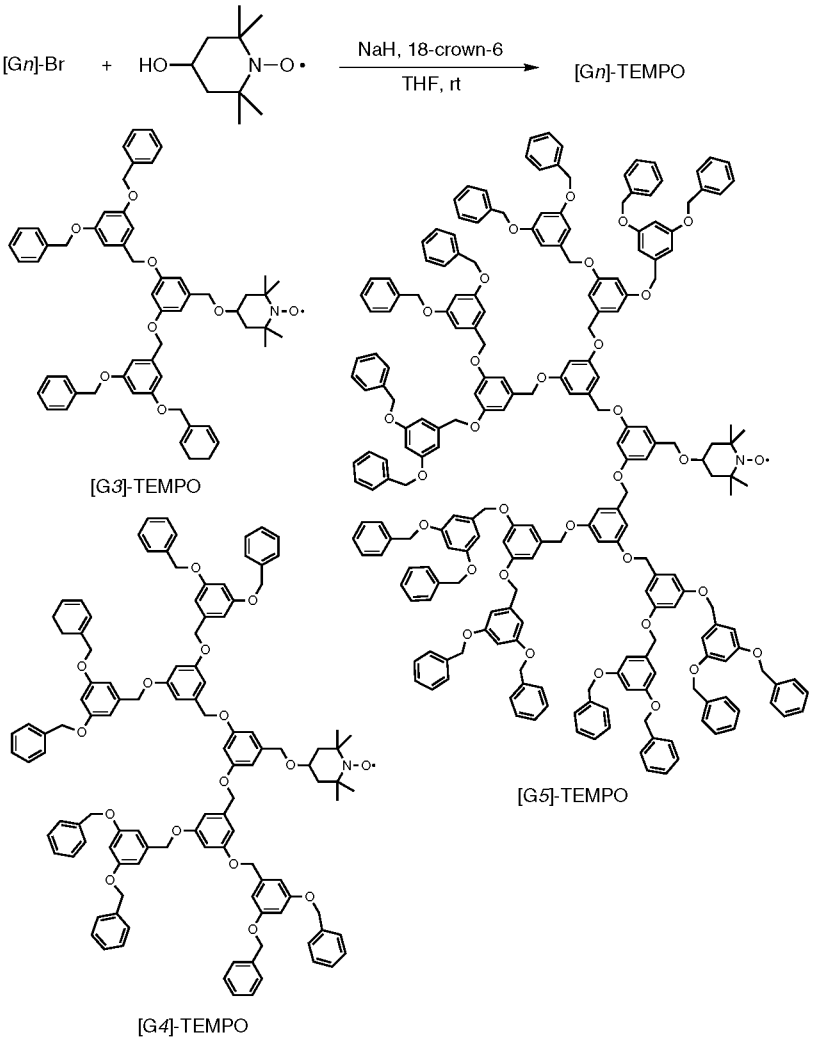
Decomposition of azo compounds and peroxides provides the alkoxyamine by the nitroxide-trapping of the primary radicals [29]. The radicals produced by hydrogen abstraction with oxy radicals are also trapped by the nitroxide [242, 243]. In the photoreaction, alkoxyamines were isolated with high yields [244]. The reactions of Grignard reagents with nitroxides [215] and the coupling reaction of sodium nitroxides with bromo compounds [234, 235] are also used. The hydrolysis of **56** followed by the reaction with acyl or alkyl halides afforded alkoxyamines with various functional groups, **63** (Eq. 68) [245–251]:



(68)



Nitroxide attached to macromolecules also induces the living radical polymerization of St. Yoshida and Sugita [252] prepared a polymeric stable radical by the reaction of the living end of the polytetrahydrofuran prepared by cationic polymerization with 4-hydroxy-TEMPO and studied the living radical polymerization of St with the nitroxide-bearing polytetrahydrofuran chain. The nitroxides attached to the dendrimer have been synthesized (Eq. 69) to yield block copolymers consisting of a dendrimer and a linear polymer [250, 253].



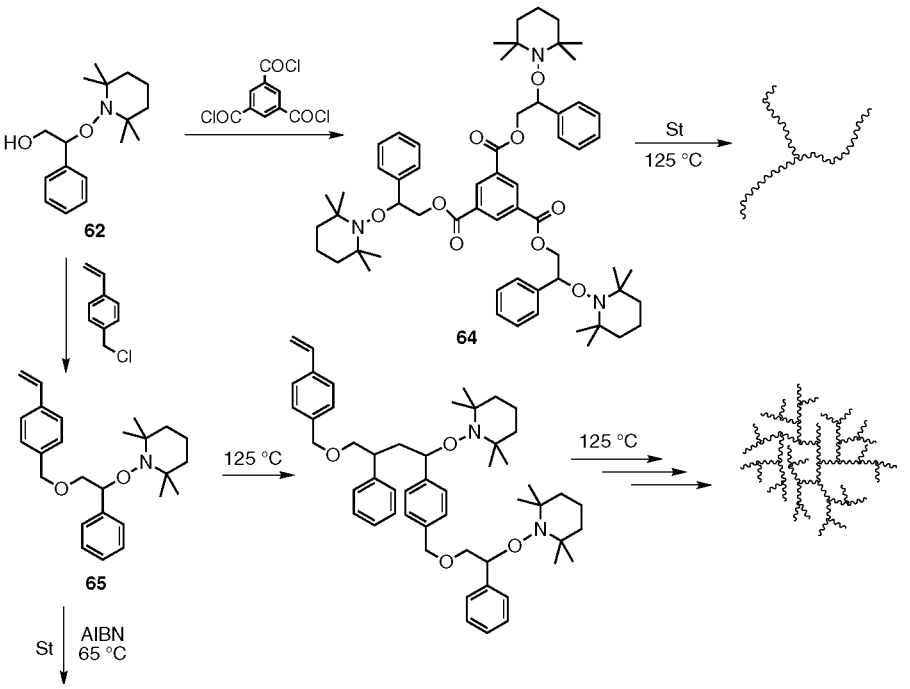
(69)

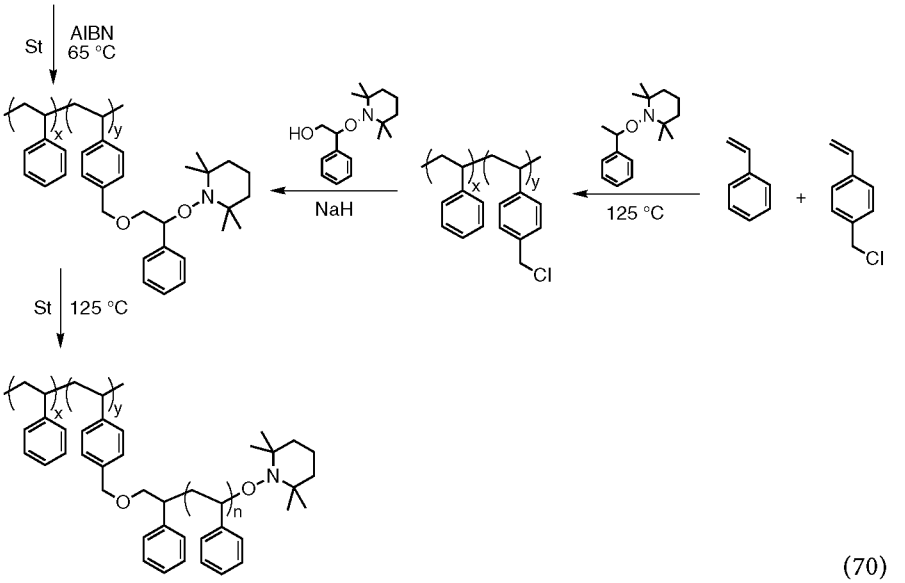
Here, G*n* represents the *n*-generation of the dendrimers. In the polymerization with these nitroxides, because the combination of the propagating radical with the nitroxide is a polymer-polymer reaction, the interaction of both polymer chains is also important, e.g., the compatibility of the poly(St) with polytetrahydrofuran or dendrimer.

The living radical polymerization of some derivatives of St was carried out. The polymerizations of 4-bromostyrene [254], 4-chloromethylstyrene [255, 256], and other derivatives [257] proceed by a living radical polymerization mechanism to give polymers with well-controlled structures and block copolymers with poly(St). The random copolymerization of St with other vinyl

monomers was investigated by several authors. In the copolymerization of St with MMA or BA, the decrease in the content of the St in the feed increased the M_w/M_n values of the copolymers produced [245]. Controlled random copolymerization was achieved in the copolymerization of St with AN, resulting in the synthesis of a diblock copolymer of poly(St) and poly(SAN) with a well-controlled structure, M_n=3.6–6.8×10^4 and M_w/M_n=1.22–1.30 [258]. The alternating copolymerization of St with *N*-cyclohexylmaleimide via a living radical polymerization with TEMPO was also examined [259]. The living radical polymerization was adopted to synthesize a fullerene end-capped poly(St), which was soluble in a variety of solvents and found to have good photoconductivity [260, 261].

The living radical polymerization with trifunctional initiator **64** provided a star polymer which had each arm the same length [247]. The monomer with both functions as the initiator and the monomer was prepared. Compound **65** provides a branched polymer through random copolymerization with St in the presence of a conventional radical initiator at a low temperature and the following living radical polymerization at 125 °C, as shown in Eq. (70). Via another path, the living radical copolymerization of St with 4-chloromethylstyrene and the subsequent polymer reaction of **62** gave a branched polymer with the controlled length of both its main chain and side chains. During the polymerization of **64**, hyperbranched poly(St) is produced without any gel formation because of the exclusive suppression of the bimolecular termination [246]:





(70)

Recently, well-defined graft copolymers have been prepared by living radical polymerization using macromonomers [262].

6.2 Living Radical Polymerization Systems with Transition-Metal Complexes

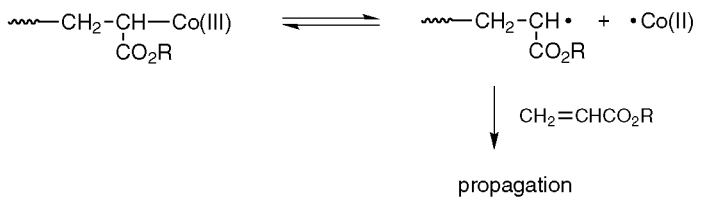
Living radical polymerizations using transition-metal complexes are divided into the following two categories: one is the use of the dormant species formed by coupling or the strong interaction of the propagating radicals to the metal complex, and the other is the carbon-halogen bond formation as the dormant species and the radical formation in combination with the metal complexes. In the late 1970s, Minoura and coworkers [60, 61] studied the polymerization of MMA with the chromium acetate/BPO initiator system, and they found that this polymerization proceeds via a living radical polymerization mechanism, but the details of the mechanism were unclear. Mun et al. [263, 264] pointed out the possibility of the living radical polymerization of MMA in the binary initiation system of cobaltocene and bis(ethylacetoacetato)copper(II). Otsu and Tazaki [153, 154] postulated that the reduced nickel induced the polymerization of vinyl monomers when it was used with alkyl halides. In these polymerizations, the molecular weight of the polymers increased with the reaction time, but the control of the molecular weight and its distribution were difficult.

<type1>In the radical polymerization of fluorine-containing olefin monomers, molecular weight control has been achieved due to the high-chain transfer constant to the carbon-halogen bond of the polymer chain end to yield polymers with a narrow molecular weight distribution and block copolymers (Iodine Transfer Polymerization) [18, 35]. In the polymerization of common vinyl monomers oth-

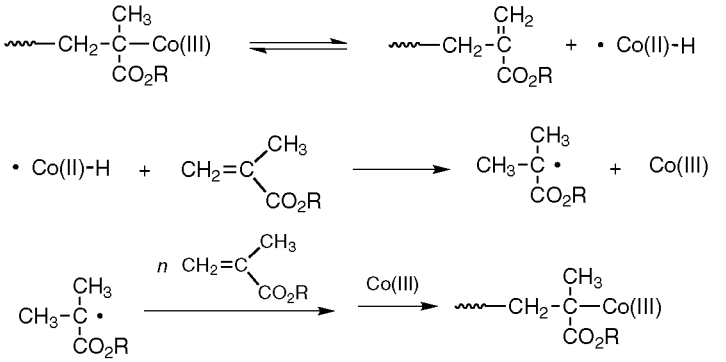
er than fluoro monomers, the molecular weight of the polymers is not controlled because the chain transfer constant is not so high [265, 266].

6.2.1
Polymerization with Carbon-Metal Bond Formation

Cobalt complexes are used for the living radical polymerization of acrylates to give a high molecular weight polymer with a narrow molecular weight distribution ($M_w/M_n \sim 1.2$) (Eq. 71), whereas the complex is applied to the introduction of an unsaturated group into the methacrylate polymers with a high efficiency via a reaction mechanism illustrated in Eq. (72) [27, 28, 267, 268].



(71)



(72)

Chromium [269, 270] or aluminum [269, 271, 272] complexes are also examined for the radical polymerization of MMA or VAc, but the reaction mechanism and the control of the polymerizations are still unclear.

6.2.2
Polymerization with Carbon-Halogen Bond Formation

Sawamoto et al. have revealed that the ruthenium complex induces the living radical polymerization of MMA [30, 273–277]. For example, $RuCl_2(PPh)_3$ provided poly(MMA) with $M_w/M_n \sim 1.1$ and the block copolymers. This system has a unique characteristic in that it is valid not only for MMA and other methacrylates, but also for acrylates and St derivatives.

Haloalkanes, haloketones, halonitriles, haloesters, and haloalkylbenzenes are used as the initiators.

Haloalkanes: CCl_4 CCl_3Br $CHCl_3$

Haloketones: CCl_3COCH_3 $CHCl_2COPh$

Halonitriles: $CH_3-\underset{\displaystyle CN}{\underset{|}{CH}}-Cl$

Haloesters: $CCl_3CO_2CH_3$ $CHCl_2CO_2CH_3$ $CH_3-\underset{\displaystyle CO_2Et}{\underset{|}{CH}}-Cl$

$CH_3-\underset{\displaystyle CO_2Et}{\underset{|}{\overset{\displaystyle CH_3}{\overset{|}{C}}}}-Br$ $CH_3-\underset{\displaystyle CO_2Et}{\underset{|}{\overset{\displaystyle CO_2Et}{\overset{|}{C}}}}-Br$ $CH_3-\underset{\displaystyle CO_2Et}{\underset{|}{CH}}-Br$ $CH_3-\underset{\displaystyle CO_2CH_3}{\underset{|}{CH}}-Br$

Halobenzenes: $CH_3-CH(Cl)-C_6H_5$ $CH_3-CH(Br)-C_6H_5$ $CH_2Cl-C_6H_5$

Di- or trifunctional initiators have also been developed to design the polymer structures including ABA-type block copolymers, and star polymers and star block copolymers.

Difunctional initiators:

$ClCH_2-C_6H_4-CH_2Cl$ $Cl_2CHCOOCH_2CH_2OCOCHCl_2$

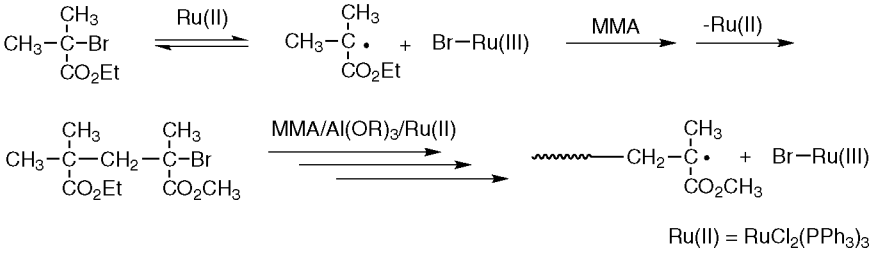
$BrCH_2-C_6H_4-CH_2Br$ $Cl_2CHCOO-C_6H_4-OCOCHCl_2$

Trifunctional initiators:

$Cl_2CHCOOCH_2$
$Et-C-CH_2OCOCHCl_2$
$Cl_2CHCOOCH_2$

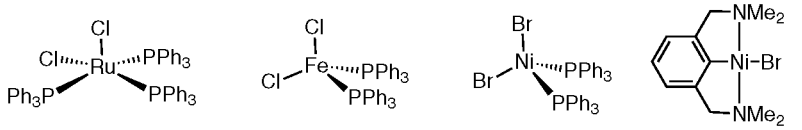
$OCOCHCl_2$
$CH_3-C(-C_6H_4-OCOCHCl_2)(-C_6H_4-OCOCHCl_2)$

The reaction mechanism is illustrated in Eq. (73):

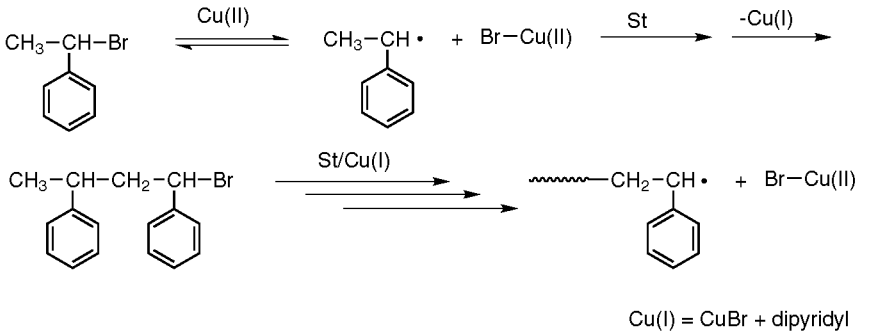


(73)

It should also be noted that this polymerization system is not disturbed in the presence of alcohol and water. Similar polymerizations with nickel [278,279] and iron [280] complexes have also been reported. The structures of the transition metal complexes are shown:



Matyjaszewski et al. [281–285] succeeded in the synthesis of poly(St) with a narrow molecular weight distribution, comparable to the living anionic polymerization, in the atom transfer radical polymerization (ATRP) using Cu complex and alkyl halides (Eq. 74):



(74)

As the initiator, a common radical initiator and arenesulfonyl chloride are also used [286, 287]. As shown in Table 6, this polymerization has a significantly large polymerization rate, and it is hardly disturbed by impurities such as alcohol and water [288]. ATRP with Cu complex was also applied to the polymerization of acrylates [289, 290], methacrylates [290–297], and AN [298] as well as St [288, 297, 299]. Because of the suppressed bimolecular termination, hyperbranched polymers are readily prepared [292], being similar to the polymerization with TEMPO previously described.

Table 6. Atom Transfer Radical Polymerization of St in the Presence of Additives[a] [288]

Additive	Time (h)	Yield (%)	M_n (Calcd)	M_n (GPC)	M_w/M_n
None	7	70	7140	5900	1.07
Ethylene carbonate	4	65	6520	7220	1.13
Water	6	60	5960	7740	1.09
Methanol	6	60	5950	7480	1.18
Acetonitrile	6	63	6300	8430	1.12
Pyridine	15	35	3480	5340	1.27

[a] Bulk polymerization at 110 °C with an additive of 5% against St. $[1\text{-PEBr}]_0 = [CuBr]_0 = [dNbipy]_0/2 = 0.087$ mol/L.

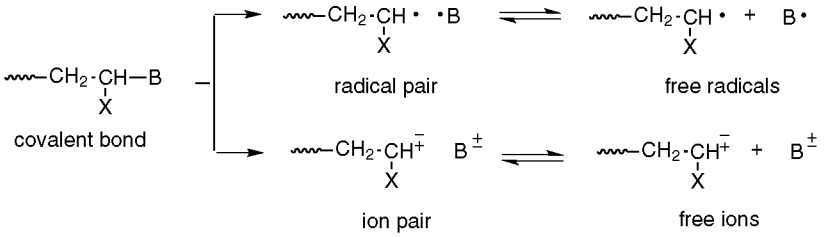
7 Conclusions

An ideal living polymerization implies only fast initiation and slow propagation processes, and the active species exist in the system because of no termination and chain transfer. Forty years have already passed since the discovery of the first living polymer by Szwarc, and the findings of various types of living polymerizations described in the introduction require a diversity in the definition and interpretation of living polymerization [3, 19, 26, 307–309]. For example, Quirk and Lee [308] defined the characteristics of living polymerization as follows: (1) polymerization proceeds until all the monomer has been consumed; further addition of monomer results in continued polymerization; (2) the number average molecular weight is a linear function of conversion; (3) the number of polymer molecules is a constant, which is notably independent of conversion; (4) the molecular weight can be controlled by the stoichiometry of the reaction; (5) narrow molecular weight distribution polymers are produced; (6) block copolymers can be prepared by sequential monomer addition; (7) chain-end functionalized polymers can be prepared in quantitative yield. They proposed the use of the terms "living polymerization with reversible termination" or "living polymerization with reversible chain transfer" for some polymerization systems.

As described in Sects 4 and 5, the iniferter technique provides a novel synthetic method for designing the chain-end structure of the producing polymers in radical polymerization. In particular, because some compounds having DC groups were found to serve as excellent photoiniferters and induce the living radical polymerization of St and MMA in a homogeneous system, these iniferter techniques have been applied to the synthesis of various tailor-made polymers, such as functional, telechelic, block, star, and graft polymers. However, there are some disadvantages, i.e., these DC photoiniferters scarcely induce radical polymerization of non-conjugative monomers, such as ethylene, VCl, and VAc. Other living radical polymerizations including nitroxides or transition-metal complexes are also valid for only some monomers, e.g., St, methacrylates, and acrylates, but not for non-conjugated monomers.

In the limiting case of Eq. (18), if radical dissociation of the iniferter bond, addition of one monomer molecule, and reproduction of the iniferter site by the

primary radical termination of the chain transfer would occur, the polymerization could proceed stepwise. In Eq. (75) the dissociation of the covalent bond of the chain end into radicals is represented, in comparison with those in an ionic mechanism. The polymerization system in which propagation consists of the homolytic insertion of a monomer molecule into the iniferter bond might be a new model for a living radical polymerization and comparable to the living systems in ionic polymerizations.



(75)

The recent developments in living radical polymerization research are really remarkable, and new discoveries are constantly being reported. A large number of papers have been published during the preparation of this article; reviews [310–315], block copolymer synthesis with iniferter [316–319], mechanism and kinetics of living radical polymerization with TEMPO [320–332], block copolymer synthesis with TEMPO [333–341] and with other stable radical [342], living radical polymerization with transition-metal complexes [343–351], block copolymer synthesis by combination of living radical polymerization with other polymerizations [352–359], theoretical studies and simulation [360–362], and applications and others [363–368]. Because this is an ever-expanding field, no one can predict the best polymerization system for the controlled polymer synthesis. It will be necessary that someone again review the studies of the living radical polymerization thoroughly and critically several year hence.

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Microgels – Intramolecularly Crosslinked Macromolecules with a Globular Structure

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List of Symbols and Abbreviations

a	exponent of Mark-Houwink equation
AIBN	2,2'-azobis(isobutyronitrile)
BD	butanediol-1,4
BuLi	butyl lithium
c/t	degree of isomerization maleic /fumaric acid units
d	polymer density
$\bar{d}_v$	volume average hydrodynamic diameter

$\bar{d}_z$	z-average hydrodynamic diameter
DHM	dodecyl hydrogen maleate
DIPB	1,4-diisopropenylbenzene
DMF	N,N'-dimethylformamide
DVB	divinylbenzene (unspecified)
1,4-DVB	1,4-divinylbenzene
1,3-DVB	1,3-divinylbenzene
t-DVB	technical DVB
DVM	divinyl monomer
E	emulsifier concentration
EP	emulsion polymerization
ECP	emulsion copolymerization
micro-EP	microemulsion polymerisation
micro-ECP	microemulsion copolymerization
EDMA	ethylene glycol dimethacrylate
EUP	self-emulsifying unsaturated polyester
GTP	group transfer polymerization
HD	hexane diol-1,6
MA	maleic anhydride
$\overline{M}_n$	number average molar mass
$\overline{M}_w$	weight-average molar mass
$\overline{M}_{w,0}$	the value $\overline{M}_w$ at zero monomer conversion
MMA	methyl methacrylate
N	number of particles
PA	phthalic anhydride
PBS	poly(*tert*-butylstyrene)
PPS	potassium persulfate
PVS	poly(4-vinylstyrene)
$Q_{v(x)}$, Q_v	equilibrium volume swelling ratio at conversion x resp. at complete conversion
$Q^0_{v(x)}$, Q^{00}_v	degree of dilution of the polymer gel in the reaction mixture at conversion x resp. at complete conversion
RCC	radical crosslinking copolymerization
R_g	radius of gyration
R_h	hydrodynamic radius
RU	residual unsaturation
S	styrene
SDS	sodium dodecylsulfate
THF	tetrahydrofuran
V_e	elution volume
W/M	water/monomer ratio (serum ratio)
x	monomer conversion
x_3	pendant vinyl group conversion
$[\eta]$	intrinsic viscosity

1 History

In polymer science and technology, linear, branched and crosslinked structures are usually distinguished. For crosslinked polymers, insolubility and lack of fusibility are considered as characteristic properties. However, insoluble polymers are not necessarily covalently crosslinked because insolubility and infusibility may be also caused by extremely high molecular masses, strong intermolecular interaction via secondary valency forces or by the lack of suitable solvents. For a long time, insolubility was the major obstacle for characterization of crosslinked polymers because it excluded analytical methods applicable to linear and branched macromolecules. In particular, the most important structural characteristic of crosslinked polymers, the crosslink density, could mostly be determined by indirect methods only [1], or was expressed relatively by the fraction of crosslinking monomers used in the synthesis.

For a crosslinking polyreaction the functionality of the monomers is the basic parameter. However, it was found long ago that, after their reaction, not all functional groups are involved in intermolecular crosslinks but also in intramolecular and cyclic links.

In the early days of polymer science, when polystyrene became a commercial product, insolubility was sometimes observed which was not expected from the functionality of this monomer. Staudinger and Heuer [2] could show that this insolubility was due to small amounts of tetrafunctional divinylbenzene present in styrene as an impurity from its synthesis. As little as 0.02 mass % is sufficient to make polystyrene of a molecular mass of 200|000 insoluble [3]. This knowledge and the limitations of the technical processing of insoluble and non-fusible polymers as compared with linear or branched polymers explains why, over many years, research on the polymerization of crosslinking monomers alone or the copolymerization of bifunctional monomers with large fractions of crosslinking monomers was scarcely studied.

Despite this situation, it was before 1935 when Staudinger and Husemann expected to obtain a soluble product by the polymerization of divinyl benzene (DVB) in presence of a solvent and expected that this product should be a colloidal molecule of a globular shape which, despite a high molecular mass, should be soluble to obtain solutions of relatively low viscosity [4]. After heating a very dilute solution of DVB for several days to 100 °C they really isolated a soluble polymer of a low viscosity in solution. The osmotically determined molecular mass was between 20|000 and 40|000. As the specific viscosity of solutions of a 'hemicolloidal' polystyrene was much lower than that of their poly-DVB, they concluded that *this polymer is a product consisting of strongly branched, 3-dimensional molecules.* As the weight-average molecular mass presumably was much higher than the number-average values obtained by osmometry, it must be concluded that Staudinger and Husemann actually obtained the colloidal macromolecules of globular shape, i.e. microgels, which they wished to prepare. But due to the inadequate methods available at this time for polymer characterization, their conclusions were not correct.

As early as 1930 microgels were considered as constituents of synthetic rubber and as the primary reaction gel in the synthesis of polybutadiene [5]. Baker [6] reviewed the early literature on microgels with emphasis on synthetic rubber. He was the first who designated microgel particles as 'new molecules' and suggested emulsion copolymerization (ECP) for localizing gelation to small dimensions.

Schulze and Crouch [7] observed that the viscosity of the soluble fraction of copolymers from butadiene and styrene decreased sharply with the conversion after an initial increase up to the point of gelation. This decrease could not be solely attributed to a selective incorporation of higher molecular mass fractions in the gel, thus leaving fractions of low molecular mass in solution. Cragg and Manson [8] reported a similar relationship between the intrinsic viscosity and the fraction of the crosslinking DVB in the ECP with styrene. Within the concentration range up to 0.1 mass % of DVB no gel was formed. Therefore, a selective removal of species with a high molecular mass could not have taken place to explain the decrease in the intrinsic viscosity observed after its increase at lower concentrations of DVB.

Shashoua and Beaman [9] prepared microgels by ECP of styrene resp. acrylonitrile with small fractions of technical DVB (t-DVB) and also other crosslinking monomers. They stated that *"each microgel particle is a single macromolecule and that the swelling forces of solvation give rise to dispersion to molecular size"*. Medalia [10] postulated that solvent dispersed microgels are thermodynamically true solutions which, according to Shashoua and Van Holde [11], may be called *microsols*.

The intrinsic viscosity of microgels described in [9] decreased with increasing fractions of the crosslinking monomer to about 8 ml/g which was still above the theoretical value for hard spheres of about 2.36 ml/g according to the Einstein equation and assuming a density of 1.1 g/ml. Obviously, due to the relatively low fraction of the crosslinking monomer, these microgels did not behave like hard spheres and were still swellable to some extent.

Sieglaff [12] prepared slightly crosslinked microgels by ECP of DVB and styrene and studied the viscosity and swelling behavior. Nicolas [13] reported on microgels in high-pressure polyethene and Heyn [14] studied microgels in polyacrylonitrile and mentioned other early works on microgels.

The history of microgels is closely related to inhomogeneous polymer networks. The first crosslinked polymer, whose structure and properties has been extensively studied, was rubber. The classical kinetic theory of rubber elasticity assumed an ideal, homogeneous network with a statistical distribution of crosslinks and network chains long enough to be treated by Gaussian statistics [15, 16]. However, in the early microgel literature the presence of microgels in synthetic rubber [e.g. 5–7] had already been mentioned as a reason for inhomogenous network structures, even in case of low crosslink densities. Later on strong experimental evidence indicated that network structures of other crosslinking polymers, such as unsaturated polyester resins, phenolic and melamine formaldehyde resins and even epoxide and isocyanate resins after curing are inhomogeneous (reviews and original literature, e.g.[17–31]).

Probably most network structures obtained by copolymerization of bifunctional monomers and larger fractions of monomers with a higher functionality

are inhomogeneous, consisting of more densely crosslinked domains embedded in a less densely crosslinked matrix, often with fluent transitions.

Besides the inhomogeneity due to a non-uniform distribution of crosslinks, other inhomogeneities due to pre-existing orders, network defects (unreacted groups, intramolecular loops and chain entanglements) or inhomogeneities due to phase separation during crosslinking may contribute to network structures [24]. It may be concluded therefore that network inhomogeneity is a widespread structural phenomenon of crosslinked polymers.

Storey [32] observed some anomalies in the dependence of the gel point at higher concentrations of DVB which suggested some inhomogeneity and a tendency to microgel formation which explained the shift of the gel point towards higher conversions.

Malinsky et al. [33] studied the copolymerization of DVB and styrene in bulk and provided further evidence of the formation of inhomogeneous structures consisting of domains of different crosslink density.

Funke et al. [34] found that on thermal curing of unsaturated polyesters (UP) and styrene the conversion of fumaric acid units decreased with an increase in temperature. A following treatment of all samples at the highest curing temperature used before, had no effect on the conversion of the fumaric acid units. By a temperature increase at an early stage of the copolymerization reaction only the reaction rate could be increased, but the final conversion was the same as that obtained after a longer time at a lower temperature.

From these results it was concluded [18] that the final crosslink density was already fixed very shortly after the beginning of the copolymerization and that a primary network was formed which determined the final network structure. Therefore, the network of cured UP-resins was considered to be inhomogeneous, consisting of domains of a higher crosslink density in a matrix of a lower crosslink density. This conclusion was supported by the fact that, unlike vulcanized rubber, samples of cured UP-resins, on swelling in thermodynamically good solvents such as benzene or chlorinated hydrocarbons, disintegrated strongly and could be easily powdered by rubbing between the fingers. Another direct support for the inhomogeneous structure of cured UP-resins came from Gallacher and Bettelheim [35] who followed the copolymerization by light scattering experiments.

These findings encouraged the synthesis of polymer networks with a well-defined inhomogeneous structure [36], using reactive microgels as multifunctional crosslinking species. Experiments of Rempp [37], who grafted living polystyrene with divinylbenzene to obtain star polymers with crosslinked centers, represented another step to preparation of inhomogeneous networks with a defined structure.

As known from Loshaek and Fox [38], substantial amounts of pendant vinyl groups remain unreacted at the end of the polymerization, especially when a larger fraction of the crosslinking monomer is used in bulk. It was close at hand, therefore, to consider the polymerization of crosslinking monomers alone in order to obtain reactive microgels. For this purpose the crosslinking reaction had to be limited to reaction volumes small enough to obtain polymer particles with a size corresponding to the stronger crosslinked domains found in

cured UP-resins. Accordingly, the method of first choice was emulsion polymerization.

For the formation of microgels the presence of a crosslinking monomer is not always necessary. Thus, microgels have also been detected in polymers prepared with bifunctional monomers, e.g. poly(acrylonitrile-co-vinylacetate) [39], polyethylene [40], poly(vinylchloride) [41] and poly(vinylidene fluoride) [42]. Obviously, the reason for the intramolecular crosslinking with the formation of microgels are side reactions.

2 Definitions

A microgel is an *intramolecularly crosslinked macromolecule* which is dispersed in normal or colloidal solutions, in which, depending on the degree of crosslinking and on the nature of the solvent, it is more or less swollen. Besides linear and branched macromolecules and crosslinked polymers, intramolecularly crosslinked macromolecules may be considered as a fourth class of macromolecules.

Though the term *microgel* has long been used and is well established, it is not quite satisfactory because it is only appropriate for the swollen state, i.e. if crosslinked macromolecules are dissolved. Moreover, *micro* refers to dimensions of more than one micrometer whereas the dimensions of microgels are usually in the range of nanometers. However, in colloquial language 'micro' is also used for something very small. Another term which has been proposed for microgels is *nanoparticles* [43]. But this name generally designates particles with dimensions in the nanometer range, irrespectively of their chemical or structural nature. Other names which have been used are *microglobules* [25], *microspheres* [44], *microparticles* [45], *microlatex* [46], *colloidal particles* and even *polymer network colloids*.

The IUPAC Commission on Macromolecular Nomenclature recommended *micronetwork* as a term for microgel [47] and defined it as *a highly ramified macromolecule of colloidal dimensions*. However, it should be noted that a *micronetwork* implies a structure and not a macromolecule or a particle, that a high ramification is not typical for these molecular particles and that the same wrong dimension is used as with *microgel*.

Because the term *microgel* has the longest tradition and is most commonly used in polymer science and technology it is reasonable to accept it as the generic term for intramolecularly crosslinked macromolecules in solution, a state in which these species of macromolecules are usually handled and characterized.

Microgels are molecular species on the border between normal molecules and particles. Contrary to linear and branched macromolecules, the surface of microgels is rather fixed, thus approaching the characteristics of solid particles. As to their size, it is somewhat difficult to define a limit because the transition from a microgel to a larger polymer particle, e.g. in coarser polymer dispersions, is gradual. Nonetheless, optical criteria related to solubility may be applied to distinguish microgels from larger polymer particles as, contrary to normal polymer dispersions, microgels form colloidal, opalescent or even clear solutions.

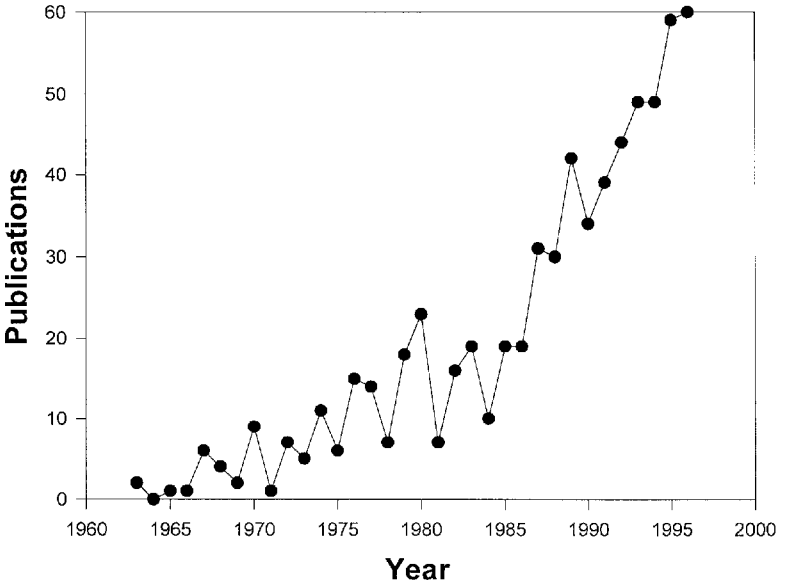


Fig. 1. Publications on microgels from 1966 until 1996 cited in Chemical Abstracts.

For a long time, microgels were rather a nuisance to the science and technology of polymers because they interfered with the characterization of macromolecules by light scattering, blocked pipes and valves in the equipment of polymer production and influenced polymer properties in an unpredictable way. Since the beginning of the 1970s, however, literature on microgels increased steadily and significantly (Fig. 1) parallel with their growing industrial and commercial importance.

Microemulsions are a convenient medium for preparing microgels in high yields and rather uniform size distribution. The name for these special emulsions was introduced by Schulman et al. [48] for transparent systems containing oil, water and surfactants, although no precise and commonly accepted definitions exist. In general a microemulsion may be considered as a thermodynamically stable colloidal solution in which the disperse phase has diameters between about 5 to 100 nm.

3 General Aspects of Microgel Synthesis

Carothers was the first who pointed out that gelation is the result of a linking process of polymer molecules into a three-dimensional network of infinitely large size [49]. The term "infinitely large size", according to Flory, refers to a molecule having dimensions of an order of magnitude approaching that of the containing vessel [50]. Thus, *such molecules are finite in size, but by comparison with ordi-*

nary molecules they may be considered infinitely large [50]. However, by decreasing the dimensions of the containing vessel, the size of the macrogel formed can be reduced. For example, crosslinking polymerization in a micelle produces a gel with a diameter of 50 nm and a molar mass of about 40×10^6 g/mol [51].

Since microgels are intramolecularly crosslinked macromolecules of colloidal dimensions, it is necessary for their synthesis to control the size of the growing crosslinked molecules. This can be achieved by carrying out polymerization and crosslinking in a restricted volume, i.e. that of a micelle or of a polymer coil. Thus, two general methods of microgel synthesis are available : (1) emulsion polymerization, and (2) solution polymerization.

According to the first method, each micelle in an emulsion behaves like a separate micro-continuous reactor which contains all the components, i.e. monomers and radicals from the aqueous phase. Thus, analogous to the latex particles in emulsion polymerization, microgels formed by emulsion polymerization are distributed in the whole available volume.

A different type of microgels can be obtained by solution polymerization. Since an increase of dilution during crosslinking increases the probability of intramolecular crosslinking, the growing polymer chains in a highly dilute solution become intramolecularly crosslinked and their structure approaches that of the microgels formed within the micelles.

Microgels prepared by these two methods exhibit different properties. Microgels, formed in an emulsion with a sufficient amount of crosslinker, behave like a macroscopic globular gel and have a similar internal structure. Unlike microgels formed in an emulsion, microgels formed in solution may have various shapes depending on the relative contributions of intra- and intermolecular crosslinking. It may be assumed, therefore, that microgels are an intermediate state of the macrogelation in solution. Figure 2 shows schematically how the polymer structure varies with the degree of dilution and the content of the crosslinker in the polymerization mixture.

In the following discussion radical crosslinking copolymerization (RCC) of mono- and bis-unsaturated monomers is considered. If a small amount of the crosslinking agent is used and equal reactivities of the vinyl groups as well as absence of cyclization are assumed, RCC would lead to a homogeneous network structure with a constant crosslink density throughout its space. However, the reactivities of vinyl groups in RCC may be different and may depend on conversion. Moreover, cyclization is possible, at least at zero monomer conversion. Therefore, inhomogeneous gel structures are always obtained, as illustrated by the Gel A, shown in Fig. 2.

If an inert good solvent is used in solution polymerization, the gel thus obtained will have a supercoiled (expanded) structure (Gel B). Gel B swells in good solvents much more than Gel A which is synthesized in bulk. If the amount of the crosslinking divinyl monomer in the reaction mixture is increased while the amount of solvent remains constant, highly crosslinked networks are formed that cannot absorb all solvent molecules present in the reaction mixture and a heterogeneous structure results (Gel C). A part of the solvent separates from the gel phase during polymerization and the formed Gel C consists of two continuous phases, a gel and a solvent phase. If the amount of solvent is further increased, a

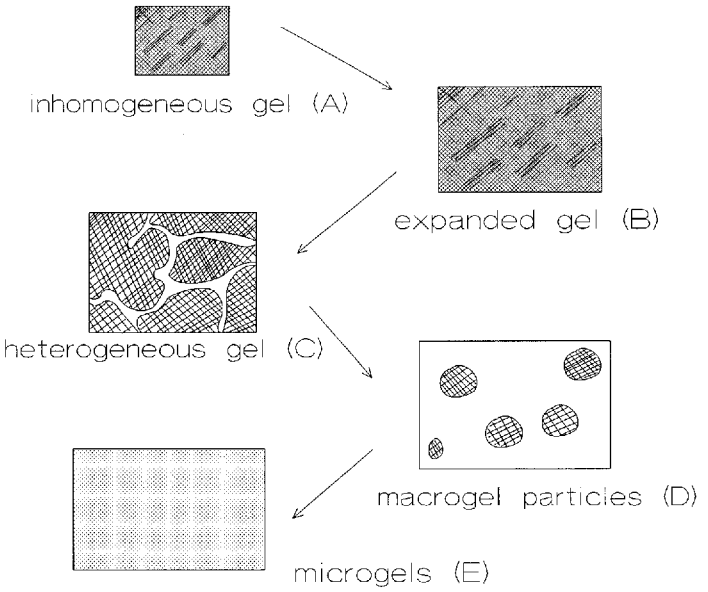


Fig. 2. Formation of various structures in radical crosslinking copolymerization of monovinyl – divinyl monomers with or without using a solvent (diluent).

critical point is passed, at which the system becomes discontinuous, because the amount of the monomer is not sufficient and the growing chains cannot occupy the whole available volume. Consequently, a dispersion of macrogel particles in the solvent results (Gel D). Increasing the amount of solvent decreases the size of the gel particles, and finally they are as small as ordinary macromolecules. These gel particles are microgels, which are dissolved as a colloidal solution (Gel E). It may be expected that at infinite dilution the macromolecules consist of intramolecularly crosslinked primary chains only which may be considered as primary particles. According to this picture of the gel formation, three main transitions can be distinguished: 1) the transition from inhomogeneous to heterogeneous gels (macrophase separation) Gel B Gel C; 2) the "solid-liquid" transition Gel C Gel D; and 3) the macrogel-microgel transition Gel D Gel E. Therefore, the preparation of microgels in RCC requires a careful choice of the experimental parameters.

It is well known that, contrary to linear or branched polymers, the structural characterization of crosslinked polymers is distinctly more difficult due to their insolubility. Since microgels prepared in emulsion behave similar to a macrogel but are soluble, they may serve as a model for the macrogels in order to study the relationships between their synthesis, structure and properties. For example, the intrinsic viscosity $[\eta]$ of the microgels can be substituted in Flory's swelling equation to estimate the crosslinking density. Phase transition phenomena which are observed in macrogels on changing external parameters can also be studied by a discontinuous change of the volume of corresponding microgels [52, 53].

Although microgels formed in a dilute solution have various structures and therefore are not as well-defined as those formed in emulsion, their characterization improved the understanding of the mechanism of gel formation in radical crosslinking copolymerization. Millar et al. showed that, in copolymerization of 1,4-DVB and styrene (S) in the presence of solvents, structures with highly crosslinked regions, so-called "nuclei", are formed which are rich in polymerized 1,4-DVB. From the surface of these nuclei a number of chain radicals grow outwardly [54]. Kast and Funke [55] and Dusek et al. [56] pointed out that the mechanism of gel formation in radical copolymerization differs significantly from the classical gelation theory [50], which assumes an initial formation of essentially linear primary molecules, followed by their linking together. According to Kast and Funke and to Dusek, intramolecularly crosslinked primary particles, i.e. microgels, may form at moderate to high concentrations of crosslinker or solvent. As the polymerization proceeds, new particles are continuously generated. However, reactions between microgels are responsible for the aggregation which leads to the formation of the macrogel [55, 56]. Macrogel formation via microgels may be described by Smoluchowski's equation [57, 58]:

$$\frac{dc_k}{dt} = \frac{1}{2} \sum_{i+j=k} k_{ij} c_i c_j - c_k \sum_{j=1} k_{jk} c_j \tag{1}$$

$$k_{ij} = i \quad j \tag{1a}$$

where c_i is the concentration of i-mer and k_{ij} is the rate constant of the interparticle crosslinking to form i+j-mers from i-mers and j-mers [59–63]. If all microgels are mutually penetrable, all functional groups are able to react, α becomes unity and, according to the Flory-Stockmayer model, gelation occurs [50, 64–66]. If only a certain fraction of the functional groups can react, e.g. those at the surface of the particles, α is less than unity.

Therefore, in a crosslinking process which is governed by the intramolecular crosslinking, the structure of the microgels is important. Currently, gel formation is qualitatively quite well understood by using the knowledge about the properties of microgels. However, a satisfactory quantitative treatment is still desirable.

4
Microgel Formation in Emulsion

4.1
Macroemulsion Polymerization

Normal emulsion polymerization is sometimes referred to as "macroemulsion" polymerization because of the large size of monomer droplets (hundreds of microns) compared to those of a "microemulsion" (tens of nanometer).

At first, the mechanism of macroemulsion polymerization of vinyl monomers [67] is shortly considered. Emulsion polymerization usually takes place in three

periods. In Period I initiation occurs where particles are nucleated. This nucleation period ends with the disappearance of the micelles. In Period II the particles grow by diffusion of monomers from droplets through the aqueous phase to and into the particles. When the monomers in the droplets have been consumed, Period III starts, in which the residual monomer in the particles and any monomer dissolved in the aqueous phase is polymerized. The end of Period III corresponds to the complete conversion of monomer to polymer. Thus, in macroemulsion polymerization the monomer is found at four locations: (i) in monomer droplets, (ii) in not yet initiated micelles, (iii) in growing polymer particles, and (iv) dissolved in the aqueous phase. As the concentration of the emulsifier increases, the amount of monomer in the droplets decreases. If the emulsifier concentration exceeds a critical value, all the monomer molecules are solubilized in the aqueous phase and the polymerization system becomes transparent which is typical for a microemulsion or a micellar solution.

Shashoua and Beaman were the first who pointed out that the emulsion polymerization of crosslinking systems is different from systems of linear polymerization [9]. They reported that there is "a tendency for the emulsion polymerization systems to coagulate during the course of polymerization. This is particularly great when high concentrations of crosslinking agent are employed" [9]. In their experiments the mol fraction of DVB isomers in the monomer mixture was less than 0.05. Kühnle and Funke synthesized reactive microgels by emulsion polymerization of 1,4-DVB and of t-DVB and determined the pendant, reactive vinyl groups by addition of mercury acetate and of BuLi [68,69]. Later on, Hoffmann prepared a series of microgels by emulsion copolymerization of t-DVB and S with amounts of DVB varying up to 17% [70]. In these experiments, an excess amount of emulsifier was used, so that monomer droplets were absent. In the following years many studies were carried out to synthesize crosslinked polymer particles, i.e. microgels, by emulsion copolymerization of vinyl/divinyl monomers [71–76].

During the past 25 years, Funke and co-workers have extensively studied the emulsion polymerization of divinyl monomers alone including 1,4-DVB and ethylene glycol dimethacrylate (EDMA) under various reaction conditions. They found that the intraparticle crosslinking changes drastically the classical picture of emulsion polymerization.

1,4-DVB (purity > 98%) was polymerized using sodium dodecyl sulfate (SDS) as emulsifier in the presence of various initiators, such as potassium persulfate (PPS) [51, 77–82], 2,2'-azobisisobutyronitrile (AIBN) [83] and also by thermal initiation [84].

Table 1. Comparison of polymer latexes obtained by emulsion polymerization of 1,4-DVB and S [79]. Experimental conditions: temperature = 50 °C; volume ratio water to monomer = 6.25, SDS concentration = 0.02 M, PPS concentration = 0.01 M. Particle diameters were measured by soap titration and by electron microscopy.

MONOMER :	$\bar{d}_z$ [nm]	10^{-15} [N / mL^{-1}]	mass % coagulum
1,4-DVB	26	13	21.7
S	57	1.5	0

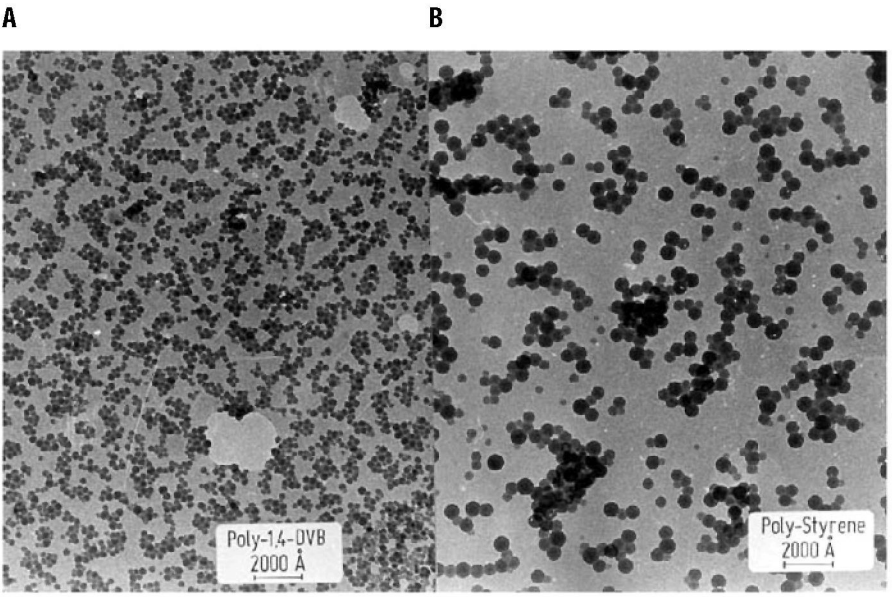


Fig. 3. Electron micrographs of polymer particles formed by emulsion polymerization of 1,4-DVB and S [79]. SDS concentration = 0.02 M, Initiator concentration = 0.01 M, temperature = 50 °C, water/monomer ratio = 6.25. [Reproduced from Ref. 79 with permission, Hüthig & Wepf Publ., Zug, Switzerland].

The polymer particles obtained by emulsion polymerization of 1,4-DVB were microgels and therefore much smaller than normal polystyrene latex particles prepared under the same experimental conditions (Table 1, Fig. 3). Table 1 shows that the average diameter of these microgels was about half of that of latex particles consisting of polystyrene. The maximum diameters of these microgels were about 50 nm. Their small particle size can be considered as a consequence of the intraparticle crosslinking which strongly restricts the swelling by monomers.

According to the classical Smith-Ewart mechanism [85], the number of particles, N, is related to the emulsifier concentration, E, by

$$N \quad E^{\nu} \tag{2}$$

where the exponent ν is predicted to be 0.6, which has been confirmed in the EP of S. However, in all experiments with 1,4-DVB at least five to ten times more particles were formed than with S [79]. The exponent ν was found to be 1.6 [80] and 1.85 [86] in the emulsion polymerization of 1,4-DVB and t-DVB respectively. When saturated polyesters instead of SDS were used as emulsifiers for the polymerization of t-DVB, the exponent ν was 1.65 [87]. Bolle showed that the exponent ν increased gradually as the fraction of 1,4-DVB in the 1,4-DVB/S mixture increased [83]. Moreover, the size distribution of microgels from 1,4-DVB is narrower than that of polystyrene latexes (Fig. 4). Another interesting property of the 1,4-DVB microgels, prepared by persulfate as initiator, is their solubility. If

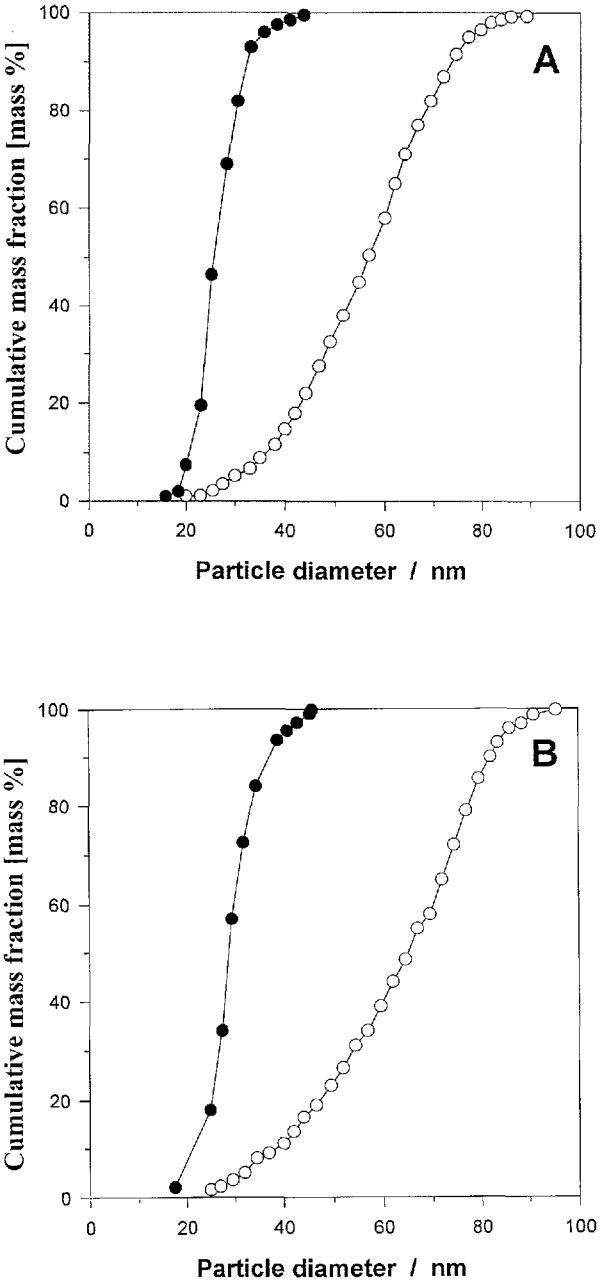


Fig. 4. Size distribution of polymer particles obtained by emulsion polymerization of 1,4-DVB ( ) and S (○). SDS concentration = 0.04 M (**A**) and 0.02 M (**B**). [Reproduced from Ref. 79 with permission, Hüthig & Wepf Publ., Zug, Switzerland].

the reaction time is sufficiently long or if a high amount of the initiator is used, the microgels become soluble in methanol [81]. Whereas latex particles and microgels prepared from styrene or DVB are completely insoluble in methanol, the addition of sulfate anion radicals to pendant vinyl groups at the surface of the microgels makes them hydrophilic and soluble in methanol.

Depending on the reaction conditions of the EP of 1,4-DVB, variable amounts of large polymer particles are formed as by-products which can easily be removed by filtration. By electron microscopy, these particles were identified as polymerized monomer droplets and as aggregates of microgels [77]. Aggregation is not surprising, because microgels may collide with each other and residual pendant vinyl groups of particles may react with radical centers of neighboring particles thus bonding them covalently together. This reaction is called interparticle crosslinking.

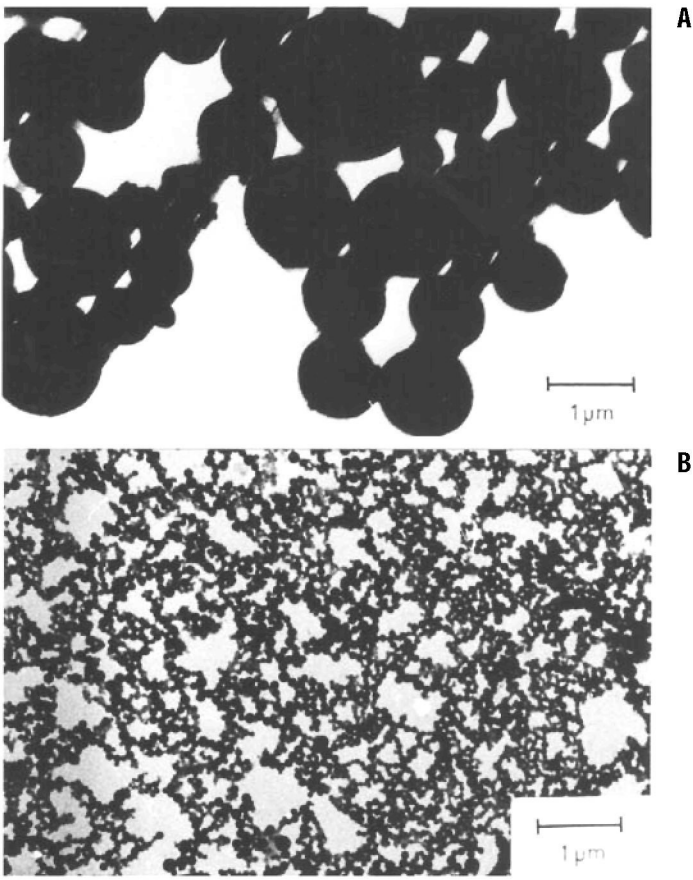


Fig. 5. Electron micrograph of the polymers formed by thermal emulsion polymerization of 1,4-DVB (A) and S (B). SDS concentration = 0.1 M, water/monomer volume ratio = 12.5, polymerization temperature = 90 °C. [Reproduced from Ref. 84 with permission, Hüthig & Wepf Publ., Zug, Switzerland].

The appearance of polymerized monomer droplets indicates that polymerization is initiated both in the monomer droplets and in monomer-containing micelles. This result is completely different from that obtained in the EP of styrene under identical conditions, where no monomer droplets polymerize. Similar experiments with 1,3,5-trivinylbenzene also yielded polymerized monomer droplets as by-products [77]. The amount of polymerized 1,4-DVB droplets further increased when PPS was replaced by an oil soluble initiator, such as, AIBN [83] or, when the EP was thermally initiated [84]. Figure 5 compares electron micrographs of the polymers formed by thermally (90 °C) initiated EP of 1,4-DVB and S.

In linear EP of bifunctional monomers, such as S, with water soluble initiators, the monomer droplets do not compete with micelles in capturing radicals from the aqueous phase because the total surface area of the droplets is much smaller than that of micelles and growing particles. Nevertheless, if some radicals enter monomer droplets, rapid termination takes place. Therefore, polymerization in monomer droplets is negligible [88]. However, if in the crosslinking EP of 1,4-DVB a few radicals are captured by monomer droplets, they can polymerize completely because the recombination of radicals is suppressed by the gel effect. Moreover, in thermal initiation or in initiation by hydrophobic initiators, such as AIBN, radicals are formed predominantly in the hydrophobic phase, i.e. in monomer droplets and in micelles, and crosslinking EP is initiated in the organic phase.

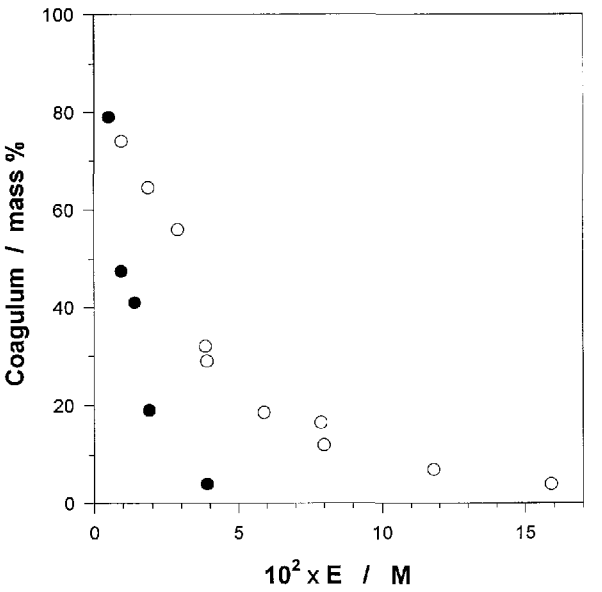


Fig. 6. Amount of coagulum as a function of the emulsifier concentration in 1,4-DVB polymerization. Polymerization temperature = 50 °C, water/monomer volume ratio = 6.25 (○), and 12.5 ( ). [Reproduced from Ref. 79 with permission, Hüthig & Wepf Publ., Zug, Switzerland].

As shown in Fig. 6, the amount of polymerized monomer droplets strongly depends on the emulsifier concentration. With increasing emulsifier concentration, the amount of monomer initially present in the monomer droplets decreases in favor of monomer solubilized in micelles. Concurrently the fraction of polymerized monomer droplets decreases and more microgels are formed. Above a certain emulsifier concentration which is about 0.8 mol/l in thermal initiation, the monomer is completely solubilized prior to polymerization and no polymerized monomer droplets are formed.

Contrary to all results known for emulsion polymerization the rate of polymerization decreases with increasing emulsifier content [83, 84] (Fig. 7). Time-conversion curves show an initial period of high polymerization rate and a subsequent period of a significantly lower rate. It seems that two parallel reactions are involved in the emulsion polymerization of 1,4-DVB: a fast polymerization in the monomer droplets and a slower polymerization in the growing microgel particles. If all monomer molecules are solubilized in the aqueous phase, i.e. at high emulsifier concentrations, the slope of the time-conversion curve changes gradually (Curve III in Fig. 7).

Spang studied the EP of EDMA under various reaction conditions and obtained similar results [89]. The differences between the crosslinking EP and EP of comonomers with similar chemical and physical properties, but different functionalities, e.g. 1,4-DVB and S or EDMA and MMA, can be explained by the

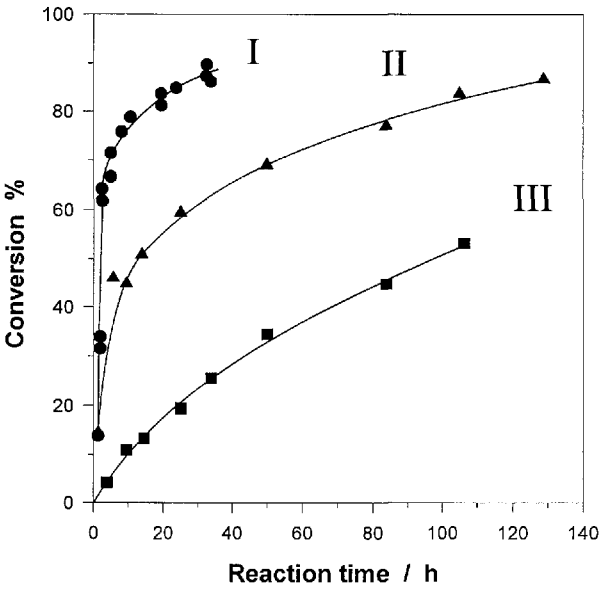


Fig. 7. Time-conversion curves of thermally initiated emulsion polymerization of 1,4-DVB at 0.1 (I); 0.65 (II); and 0.85 (III) M SDS concentrations. Polymerization temperature = 90 °C; water/monomer volume ratio = 12.5. [Reproduced from Ref. 84 with permission, Hüthig & Wepf Publ., Zug, Switzerland].

characteristics of radical crosslinking emulsion polymerization. These characteristics which are due to the network formation, are:

- formation of significantly more and smaller monodisperse polymer particles;
- polymerization of monomers in the monomer droplets as well as in the polymer particles;
- decrease in the polymerization rate with increasing emulsifier concentration.

In the following a possible mechanism of microgel formation in crosslinking EP, using water soluble initiators, is given [79, 84, 90, 91].

Radicals or oligomer radicals are generated in the aqueous phase and enter monomer-swollen micelles and initiate polymerization and crosslinking to form microgels. Polymer particles formed from vinyl monomers consist of 50–70% monomers and, until the end of Period II, i.e. as long as monomer droplets are present, the monomer concentration in the polymer particles remains almost constant. However, in crosslinking EP the network formation of microgels limits the amount of absorbable monomer, thus also limiting their growth. It must be noted that in EP the molar mass of the growing polymer chains is much higher than in bulk polymerization because of the compartmentalization in the particles. Because the primary polymer molecules are long and since the reactions within the polymer particles occur under bulk conditions, one may expect a very early onset of macrogelation within the particles, i.e. already during Period I. Recent calculations also show that in crosslinking EP of tetrafunctional monomers the crosslink density is very high from the very beginning of the reaction, so that the absorption of monomer by the polymer particles is restricted even in Period I [92]. Beyond the gel point, the decrease of the monomer concentration in the polymer particles will enhance the probability of multiple crosslinking, so that the crosslinking density of the particles will increase very rapidly and a tighter network structure results. This also reduces the growth rate of the polymer particles and the size of the particles.

During the period of particle nucleation in the EP of vinyl monomers, usually one of every 100–1000 micelles captures a radical and becomes a polymer particle. All other micelles give their monomers and emulsifier molecules to neighboring micelles which have captured a radical. However, since the growth rate of polymer particles decreases by crosslinking, monomer-containing micelles exist for a longer time and therefore have a better chance to capture radicals for polymerization. As a result, more polymer particles are produced in crosslinking than in linear EP.

Due to the reduced absorption of monomers and the low rate of polymerization in the micelles, the diffusion of monomer molecules from droplets to the growing particles is limited. Correspondingly, the probability of polymerization in the droplets increases.

In EP of bifunctional vinyl monomers, the reaction rate increases with the emulsifier concentration because the number of particles increases. However, in the crosslinking EP of divinyl monomers, the reaction rate is inversely proportional to the emulsifier concentration. This unusual behavior is due to nucleation taking place in both micelles and monomer droplets. In monomer droplets, the kinetics resembles that of bulk polymerization and therefore the reaction rates

are higher than in micelles. As the amount of monomers available for the polymerization in the monomer droplets is determined by the emulsifier concentration, an increase of the emulsifier concentration decreases the amount of the monomer in the droplets and accordingly the rate of polymerization also decreases.

4.2 Microemulsion Polymerization

A more efficient way to synthesize microgels is microemulsion polymerization (micro-EP). Three characteristics distinguish micro-EP from EP [93, 94]: (1) no monomer droplets exist but only micelles or microemulsion droplets which are probably identical; (2) the initiator stays in the microemulsion droplets only and polymerization occurs only there, provided oil-soluble initiators are used; and (3) the reaction mixture is optically transparent and in an equilibrium state. Compared to EP, polymerization in a microemulsion is a very simple method for the controlled synthesis of microgels because monomer droplets are absent. Using micro-EP, Antonietti et al. prepared spherical microgels with diameters of 60–170 nm by copolymerization of 1,3-diisopropenylbenzene and S, using a combination of a derivative of a polyethylene oxide as a polymeric emulsifier and sodium dodecyl sulfate (SDS) [95]. Bolle studied the micro-ECP of 1,4-DVB and S using only SDS and synthesized a series of microgels with different diameters and degrees of swelling [83].

4.3 Characteristic Properties of Microgels

Since a microgel is a solvent-containing three-dimensional macromolecule, its mass in the dry state may be compared with the mass of a polymer particle formed in EP. Accordingly, each factor that influences the size of monomer-containing species also influence the molar mass of a microgel. Figure 8 shows how the weight-average molar mass $\overline{M}_w$ of 1,4-DVB microgels and their hydrodynamic diameter $\overline{d}_z$ in toluene vary with the emulsifier (SDS) concentration [83]. Both $\overline{M}_w$ and $\overline{d}_z$ decrease with an increase of the emulsifier concentration because the size of the micelles is decreased. This decrease is first rapid but then slower at a SDS concentration of about 0.6 mol/l, where all 1,4-DVB molecules are solubilized in micelles [83].

Due to the compact structure of microgels, their intrinsic viscosities, $[\eta]$, are much smaller than those of corresponding linear or branched polymers. In Fig. 9, $[\eta]$ of DVB-microgels is plotted against their crosslink density in terms of the mol % of crosslinking monomer in the initial monomer mixture. The experimental data points were taken from different sources [9, 12, 70, 83, 95]. Though both the conditions of synthesis and measurement and the kind of monomers differed, the results can be represented by a single curve. $[\eta]$ first decreases strongly up to about 3% of crosslinking monomers, and finally attains a limiting value of 4 ml/g which is somewhat higher than the value for rigid spheres 2.3 ml/g of the Einstein equation for viscosity. For EDMA microgels formed by EP, $[\eta]$ in

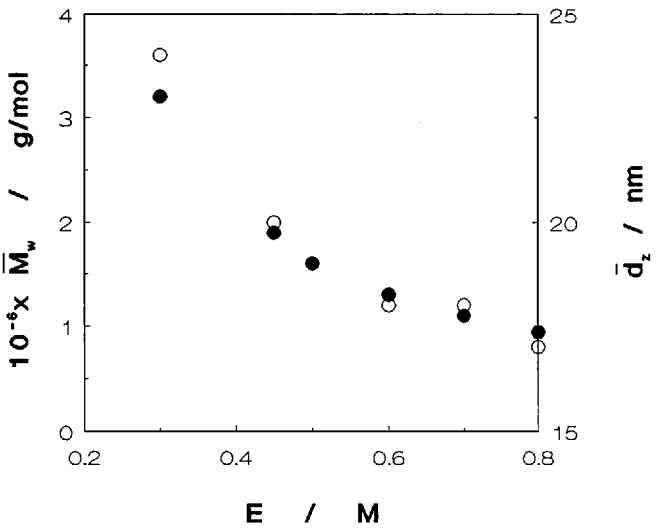


Fig. 8. Variation of the weight-average molar mass $\overline{M}_w$ ( ) and z-average hydrodynamic diameter in toluene $\bar{d}_z$ (○) with the emulsifier concentration in the emulsion polymerization of 1,4-DVB [83]. Polymerization temperature = 70 °C, initiator = AIBN, water/monomer ratio = 12.5.

n-butyl acetate or in dioxane was also 4 ± 1 ml/g [89]. It seems that this is a limiting value of [η] for microgels which corresponds to a volume swelling ratio of 1.8.

Accordingly, microgels swell a little in good solvents, of course, depending on their crosslink densities. Shashoua and Beaman [9], Hoffmann [70], and Antonietti et al. [95] showed that the swelling ratios of microgels, calculated from their [η], agree with the swelling ratios of macrogels. This would mean that microgels qualitatively obey the theory of rubber elasticity. By applying Flory's swelling equation, the calculated crosslink density of microgels is lower than that expected from their composition due to an inefficient crosslinking [95]. It was also shown that, like with macroscopic gels, the dependence of the degree of swelling on the solubility parameter of the swelling agent can be used to estimate the solubility parameter of the microgels [12].

Figure 10 shows the variation of the exponent a of the Mark-Houwink equation with the 1,4-DVB content of microgels. The measurements were carried out at 25 °C in salt-containing *N,N'*-dimethylformamide (DMF) (<10 mass % of 1,4-DVB) or in toluene (>10 mass % of 1,4-DVB) [70, 83]. The exponent a is close to zero for 1,4-DVB contents higher than 0.3 mass % and becomes zero above 10 mass % of 1,4-DVB. At low crosslinker contents one may expect that the network chain ends, emerging from the microgel surface, may lead to the observed slight molar mass dependence [η]. However, for 1,4-DVB contents higher than 10 mass %, microgels in solution behave like homogeneous gel spheres with a constant density.

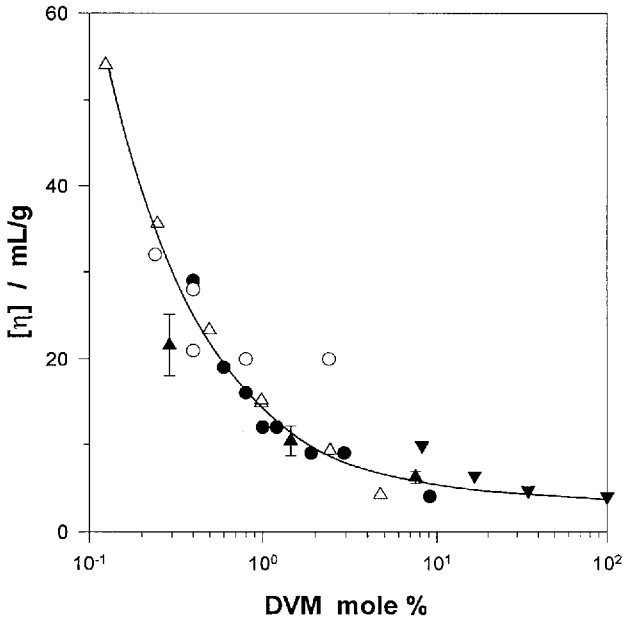


Fig. 9. Variation of the [η] of microgels formed by emulsion polymerization with the amount of divinyl monomer (DVM) in the monomer mixture. The experimental data points were taken from following sources:

( ): Shashoua and Beaman [9]; t-DVB/S microgels; initiator = PPS; measurements in benzene at 30 °C.

(○): Sieglaff [12]; t-DVB/S microgels; initiator = PPS ; measurements in toluene at 25 °C.

(▲): Hoffmann [70]; t-DVB/S microgels; initiator = PPS; measurements in salt-containing DMF at 25 °C. Average values of microgel fractions were taken. The error bars indicate the standard deviations.

(△): Antonietti et al [95]; 1,3-diisopropenylbenzene/S microgels; initiator = AIBN; measurements in toluene at 20 °C.

(▼): Bolle [83]; 1,4-DVB/S microgels; initiator = AIBN; measurements in toluene at 25 °C.

Since the radius of gyration, R_G, is sensitive to refractive index distribution (mass distribution) within the polymer coil, while the hydrodynamic radius, R_H, is sensitive to the flow properties, the ratio R_G/R_H also informs us about the inner structure of microgels. For a random coil in a Θ-solvent this ratio is 1.73 while for a hard sphere of uniform density it is 0.775 [96, 97]. For various microgels prepared in emulsion, the R_G/R_H ratio was found to be smaller than that for a rigid sphere and approaches its ratio with increasing crosslinker content [73, 95–98]. These measurements also indicate that the microgels with low crosslink densities have a non-uniform polymer segment density, whereas those with a high crosslink density behave like homogeneous spheres.

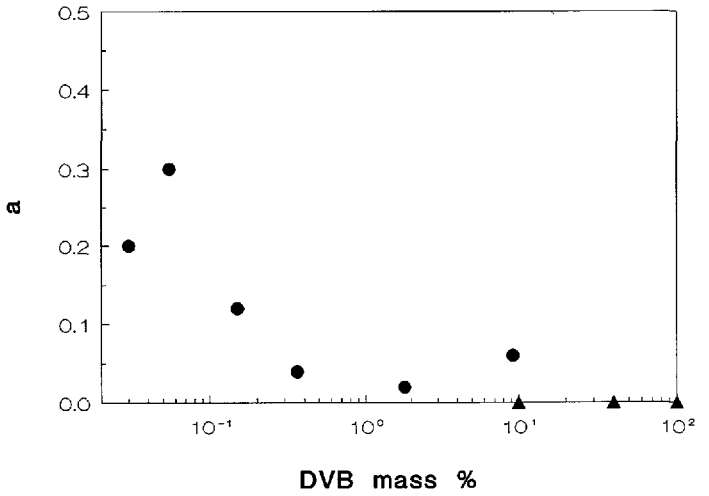


Fig. 10. Dependence of the exponent a of Mark-Houwink equation on the 1,4-DVB content of the microgels formed in emulsion. The data points were calculated from the $[\eta]$ and $\overline{M}_w$ values reported by Hoffmann ( ) [70] and by Bolle (▲) [83].

4.4 Expanded (Preswollen) and Heterogeneous (Porous) Microgels

Heterogeneous (porous) macrogels are widely used as starting materials for ion exchangers and as specific sorbents. Therefore, the mechanism, with which these structures are formed by copolymerization of divinyl/vinyl monomer mixtures has been the subject of many studies [e.g. 54, 99–106]. It is interesting to compare these results with those obtained using microgels, though only a few experiments with microgels have been reported [70, 95].

Depending on the conditions of synthesis, copolymerization of divinyl/vinyl-monomers in the presence of an inert solvent leads to the formation of expanded (preswollen) or heterogeneous (porous) structures [54, 99, 100]. If the solvent remains in the network (gel) phase throughout the copolymerization, expanded networks are formed. If the solvent separates from the network phase the network becomes heterogeneous. According to Dusek et al., heterogeneities may appear in poor solvents due to the polymer-solvent incompatibility (χ-induced syneresis), while in good solvents due to an increase in crosslink density (ν-induced syneresis) [99].

Now the post-gelation period of the copolymerization of divinyl/vinyl monomers in the presence of a good solvent as a diluent will be considered. Let $Q_{v(x)}$ be the equilibrium volume swelling ratio of the gel formed at conversion x, and $Q^0_{v(x)}$ its degree of dilution in the reaction system, i.e.,

$$Q^0_{v(x)} = \frac{\text{volume of swollen gel in (monomer + solvent) mixture}}{\text{volume of dry gel at conversion x}} \qquad (3)$$

Both $Q_{v(x)}$ and $Q^{0}_{v(x)}$ decrease as the polymerization proceeds and, after a definite conversion $Q_{v(x)}$ may reach the value of $Q^{0}_{v(x)}$. Since the dilution of a gel cannot be greater than its equilibrium degree of swelling, the excess of solvent should separate from the gel phase resulting in the syneresis, i.e. in phase separation. The condition for incipient phase separation during copolymerization of divinyl/vinyl monomers is given by [107]

$$\frac{Q_{v(x)}}{Q^{0}_{v(x)}} \quad 1 \tag{4}$$

Assuming a homogeneous distribution of crosslinks, the equality, given by Eq. (4), becomes independent of conversion. Thus on complete conversion (x = 1), $Q^{0}_{v(x)}$ reduces to Q^{00}_{v} (initial degree of dilution of the monomers) and $Q_{v(x)}$ can be replaced by the experimentally determined equilibrium swelling ratio Q_v. Accordingly, the condition of phase separation becomes

$$\frac{Q_v}{Q^{00}_{v}} \quad 1 \tag{5}$$

The experimental data obtained with macrogels formed in the presence of solvents, agreed well with Eq. (5) [99, 105, 108]. In order to check the applicability of this equation to microgels, the experimental data reported by Hoffmann [70] are used. He prepared a series of microgels with different crosslink densities, using toluene as a solvent, at $Q^{00}_{v} = 5$. Q_v was calculated from the reported data using the equation $Q_v = \frac{[\ ]d_p}{2.5}$ and assuming the density of the polymer as $d_p = 1.1$ g/ml [83]. The normalized swelling ratio of the Hoffmann's microgels is given by $[\eta]/[\eta]_0$ where $[\eta]$ and $[\eta]_0$ are the intrinsic viscosities of the microgels prepared with and without using a solvent respectively.

Figure 11 illustrates the Q_v/Q^{00}_{v} ratio and the normalized swelling ratio $[\eta]/[\eta]_0$ plotted as a function of the 1,4-DVB content of the monomer mixture. For Q_v/Q^{00}_{v} values greater than unity, the microgels prepared in the presence of toluene swell twice as much as those prepared without a solvent . Thus, these microgels have an expanded (supercoiled) structure. Like in macrogels, the swelling ratios do not depend on the crosslinker content. However, if the ratio Q_v/Q^{00}_{v} of microgels drops below unity, the swelling ratio decreases simultaneously, which indicates the onset of phase separation within the microgels during polymerization and the appearance of heterogeneities. Since toluene separates from the gel phase, the swelling ratio approaches that of microgels formed without a solvent. As seen in Fig. 11, the incipient phase separation within the microgel particles occurs at about 6 mass % of 1,4-DVB. This value of a critical DVB concentration is reasonable considering reported values for t-DVB/S macrogels formed in toluene. Millar et al. reported critical DVB concentrations of 30 and 15 mass % t-DVB for $Q^{00}_{v} = 1.5$ and 4.0 respectively [54]. Although the experi-

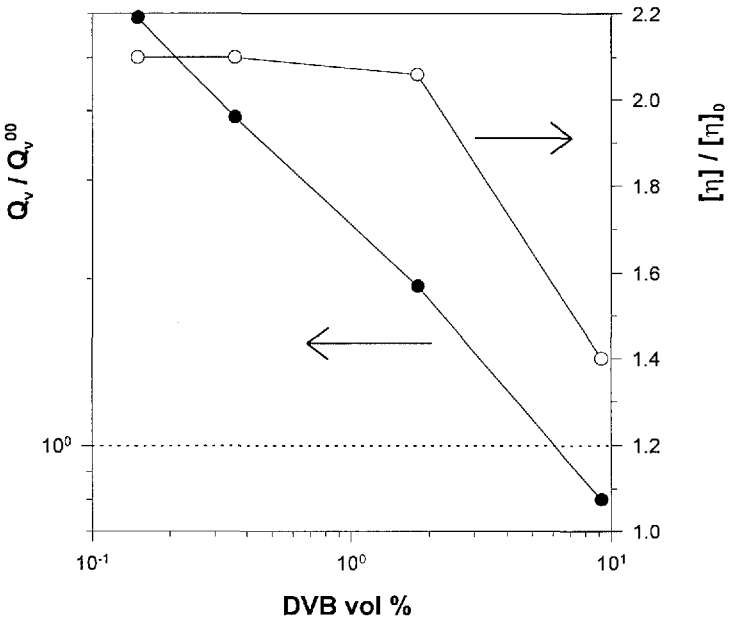


Fig. 11. Variation of Q_v/Q_v^{00} ratio ( ) and the reduced intrinsic viscosity of microgels $[\eta]/[\eta]_0$ (○) with the DVB content in the monomer mixture. Experimental data points were taken from Hoffmann [70]. The dotted horizontal line represents the critical Q_v/Q_v^{00} value for the onset of a phase separation.

ments, carried out in the presence of solvents are incomplete and more experimental evidence is necessary, these experiments and calculations demonstrate the formation of preswollen and heterogeneous microgels.

5 Microgels by Emulsion Copolymerization of Self-Emulsifying Unsaturated Polyesters and Comonomers

By emulsion copolymerization (ECP) of self-emulsifying unsaturated polyesters (EUP) and bifunctional monomers, such as styrene (S), microgels may be prepared which have a rather uniform diameter [109]. This uniformity of size is due to a special mechanism of particle formation involved in using EUP as comonomers.

Unsaturated polyesters that are terminated by carboxylic acid groups at both ends of the chain after neutralization are efficient emulsifiers for lipophilic monomers [110] and thus act as self-emulsifying crosslinking agents in the ECP of these systems. Normal emulsions of EUP and comonomers have a white, milky appearance. With an appropriate structure and molar mass of the EUP and within a certain range of EUP/comonomer ratios, however, microemulsions are

obtained [111] which are opaque or almost clear. If EUP/comonomer mixtures are copolymerized in such microemulsions, high yields of microgels result without formation of insoluble coagulates or agglomerates.

For preparing microemulsions, normally larger amounts of an external emulsifier, if not other additives, are needed. Both have to be removed after the reaction. Self-emulsifying copolymerization of EUP and comonomers in a microemulsion (micro-ECP) avoids these disadvantages. Moreover, besides the emulsifying and crosslinking function, the EUP provides carboxylic acid groups at the surface of the microgels that may be used for further chemical modifications or for crosslinking with other reactive compounds or macromolecules.

By using lipophilic initiators, such as 2,2'-azobis(isobutyronitrile) (AIBN), in the micro-ECP, diffusion of monomers is too slow compared with the reaction rate. Therefore, copolymerization is confined to the incoherent, lipophilic phase [112, 113] and very small microgel particles with a rather uniform size result.

5.1
Unsaturated Polyesters as Self-Emulsifying Components of Copolymerization

Unsaturated polyesters with neutralized terminal carboxyl acid groups (EUP) are efficient emulsifiers which, at a sufficient concentration, may form aqueous microemulsions. Microemulsions are liquid dispersions of translucent (opalescent or transparent) appearance. Their disperse phase contains particles of diameters between 20 and 80 nm which closely approaches the diameters (5–15 nm) of micelles [114].

In aqueous dispersions of EUP the diameters were found to be about 5–25 nm and the corresponding dispersions of these EUP and comonomers up to about 50–60 nm [115]. Accordingly, these dispersions may be classified as microemulsions.

For the self-emulsifying function of EUP, its molar mass should be within certain limits which depend on the molecular structure of EUP. With higher molar masses normal emulsions are formed and, depending on the solubilization procedure of the lipophilic monomers, normal or multiple emulsions may be obtained [111]. Moreover, the degree of isomerization, *cis/trans* (c/t) is important for the solubilizing property and the reactivity of the EUP. For acting as emulsifiers the terminal acid groups of the EUP must be neutralized by inorganic or organic bases, such as NaOH or tertiary amines.

Because the conditions of solubilization and copolymerization of EUP/comonomer systems as well as the characteristics and properties of the microgels depend on a variety of parameters, these data are included in the following figures and their captions.

5.1.1
Solubilization of the Monomer Mixture

The sequence of dispersing the EUP and the lipophilic comonomer in water profoundly influences the structure of the emulsion obtained. If the EUP is first dissolved in the comonomer and then this mixture dispersed in water containing

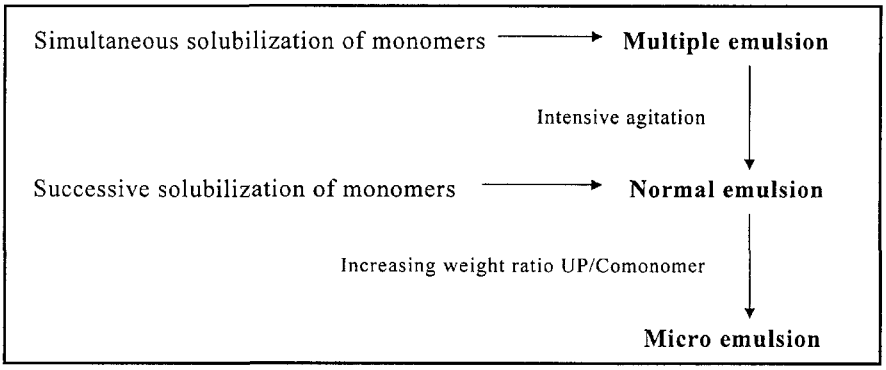


Fig. 12. Preparation of different emulsions of self-emulsifying unsaturated polyesters (EUP) and comonomers.

the base needed for neutralizing the carboxylic acid groups of the EUP, multiple emulsions are obtained. Only by very efficient agitation, such as ultrasonic treatment, do multiple emulsions gradually change to normal emulsions (Fig. 12). This indicates that diffusion processes in mixing of a colloidal systems may be much slower than in mixing components of normal solutions.

By first dispersing the EUP in water containing the base for neutralization of the carboxyl acid groups of the EUP and then adding the comonomer with intensive stirring, normal emulsions are obtained. They are favorable because, with multiple emulsions, insoluble polymers are formed, which decrease the yield of microgels.

For self-emulsification the molar mass of the EUP must be within a certain range. If the molar mass is too high, the solubility of the EUP is too low. If the molar mass is too low, the solubilizing efficiency is insufficient. With an EUP from maleic anhydride (MA) and hexanediol-1,6 (HD) and acid terminal groups, the optimal molar mass for the solubilization of a hydrophobic comonomer, such as styrene (S), was found to be between about 1700 and 2200 [116].

For studying the emulsifying properties, saturated polyesters can be used to avoid complications by the reactivity of unsaturated units of the EUP [117]

5.1.2
Critical Micelle Concentration of Unsaturated Polyesters

Like other emulsifiers, an EUP forms micelles at a critical micelle concentration (CMC). For comonomer-free EUP-emulsions of the (MA+HD)- type the CMC is about 5×10^{-4} g/ml [115, 118]. The CMC depends on the composition and chain length of the polyester, the presence of an electrolyte [118] and the temperature.

An increase in the molar mass of EUP decreases the CMC (Fig. 13), but this effect almost disappears at higher molar masses. With higher molar masses, less EUP molecules are needed for micelle formation, but this tendency is limited by the required solubility of the EUP in water.

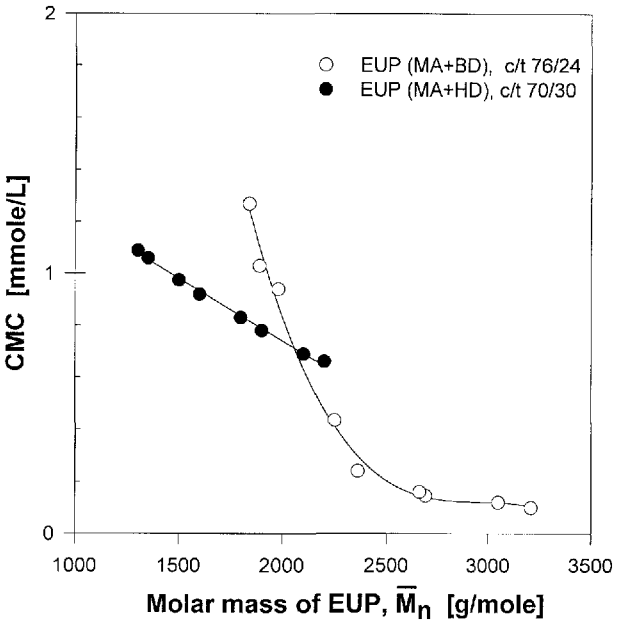


Fig. 13. Relation between the critical micelle concentration (CMC) of self-emulsifying unsaturated polyesters (EUP) and their $\overline{M}_n$ [119, 120].

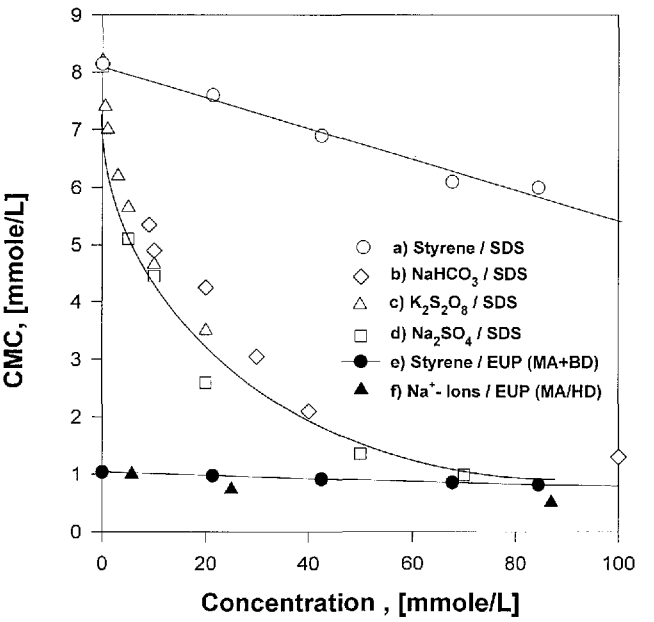


Fig. 14. Relation between the CMC of SDS and EUP. a) – e): [119], f): [118].

Electrolytes strongly decrease the CMC of usual emulsifiers, such as sodium dodecyl sulfate (SDS) (Fig. 14). The source of electrolytes in an emulsion polymerization may be carboxylate groups terminating the EUP molecules, radical initiators (e.g. $K_2S_2O_8$), inorganic bases (e.g. $NaHCO_3$) for neutralizing acid degradation products of persulfate initiators or other external electrolytes. With an EUP, the effect of electrolytes, such as Na^+-ions, on the CMC is much less pronounced than in case of SDS. The presence of hydrophobic comonomers, such as S, decreases the CMC. This decrease is smaller with EUP- than with SDS-micelles. The nature of the cation also plays a role for CMC.

With increasing temperature the CMC passes through a minimum (Fig. 15). The initial small decrease at low temperatures is due to a positive enthalpy of the micelle formation whereas the stronger increase of CMC towards higher temperatures is caused by a thermal perturbation of the emulsifier molecules in the micelles. The smaller influence of the temperature on the CMC in case of EUP indicates that these micelles are thermally more stable than SDS-micelles.

5.1.3
Micelles and Microemulsion Droplets

The incorporation of comonomers increases the mean hydrodynamic diameter of EUP-micelles, $\bar{d}_z$ (Fig. 16). Contrary to CMC, the $\bar{d}_z$ of micelles resp. microemulsion droplets increases with the concentration of an external electrolyte.

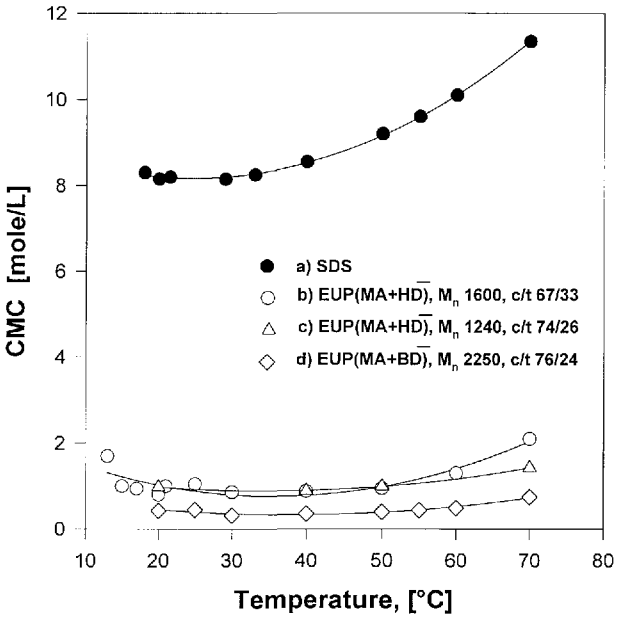


Fig. 15. Dependency of CMC of SDS and various EUP on temperature a) + d): [119], b): [120], c): [118].

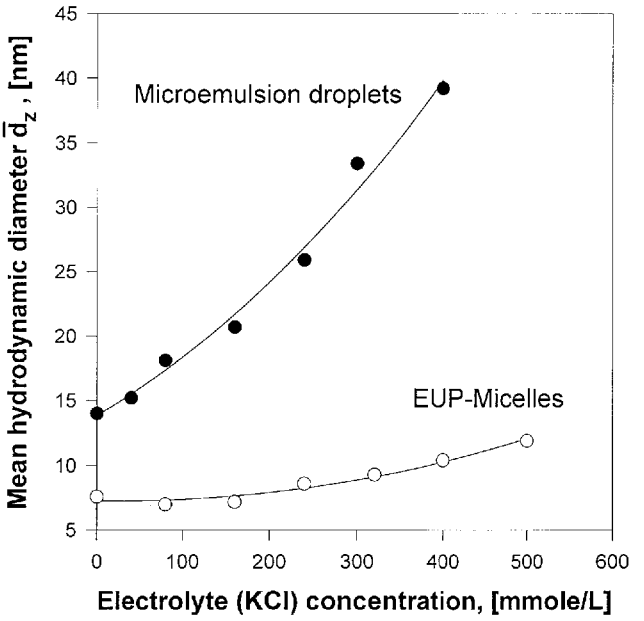


Fig. 16. Influence of electrolyte (KCl) on $\bar{d}_z$ of EUP-micelles and EUP/S-microemulsion droplets [122]. EUP(MA+HD), $\overline{M}_n$ 1290, c/t 80/20, EUP/S 4, pH 7.5, W/M 25.

However, this increase is much more significant in case of the microemulsion droplets than of EUP-micelles. The volume of a EUP-micelle increases by a factor of 6 when S is added in the mass ratio EUP/S of 80/20 and the volume of such a microemulsion droplet increases once more by a factor of 2 when 100 mmol of KCl are added.

Towards high concentrations of the electrolyte, the microemulsion changes to an emulsion containing normal monomer droplets. With a further increase in the electrolyte concentration, the emulsion becomes unstable and breaks down ("salting out").

Considering the diameters of both disperse species, the transition from micelles, containing comonomers, to microemulsion droplets seems to be rather continuous. It is therefore questionable whether a distinction between both species is justified.

By choosing a suitable structure of the EUP, not using a large excess of the base for neutralizing the carboxyl acid end groups and by applying a low temperature, a significant hydrolytic degradation of the polyester during solubilization and copolymerization can be avoided.

Hydrophobic solubilizates such as styrene (S) decrease the saponification rate of the EUP. Accordingly, the EUP-molecules in micelles containing S are more resistant against hydrolytic degradation than molecularly dissolved EUP-molecules. Obviously, the access of the base to the hydrophobic interior of these micelles and microemulsion droplets is more difficult.

5.2 Emulsion Copolymerization of Self-Emulsifying Unsaturated Polyesters and Comonomers

In normal emulsion polymerization the diffusion of monomers from droplets allows particles to grow. The polymerization is usually initiated in the aqueous phase and the oligomeric radicals either enter micelles or merge with other growing species. In the crosslinking ECP of EUP the ratio EUP/comonomer and the solubility or insolubility of both components and the initiator in the aqueous and non-aqueous phases respectively are parameters which decide whether diffusion of the reactants in the aqueous phase plays a role and where the initiation takes place.

Emulsion copolymerization of EUP and comonomers may be initiated in the aqueous (persulfate) or in the non-aqueous phase (AIBN). On the decomposition of persulfates, sulfate and hydroxyl groups are introduced into macromolecules and microgels, thus influencing their surface properties [118, 123–125]. By using AIBN as initiator a change of the chemical character of the surface and of the properties of the microgels is avoided.

Apart from the kind of components used in preparing microgels from EUP and comonomers, the yield essentially depends on the composition of the reactive components, on the water/monomer ratio, the W/M (serum ratio), the degree of neutralization of the EUP [91] and on the concentration of electrolytes.

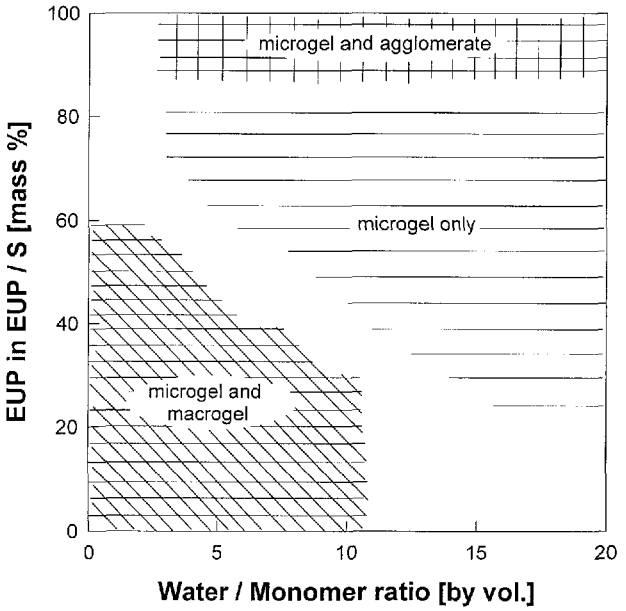


Fig. 17. Product profile of ECP of EUP(MA+PA+HD) and S [110].

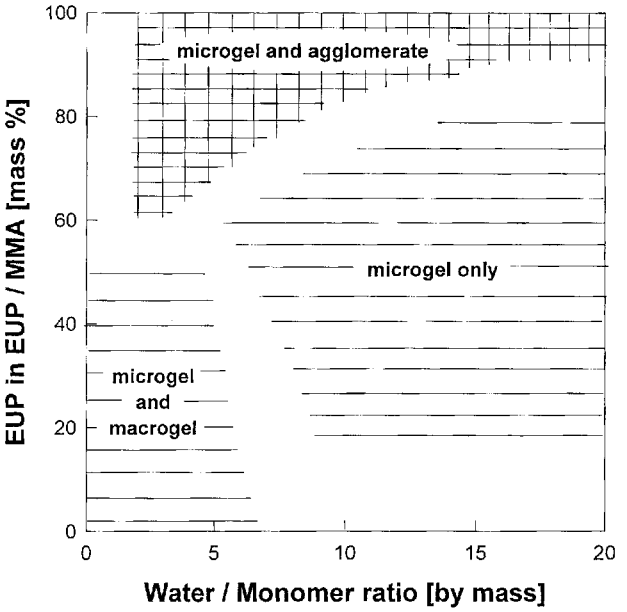


Fig. 18. Product profile of ECP of EUP(MA+PA+HD) and MMA [126].

Yields of microgels may be impaired by the polymerization of monomer droplets with formation of insoluble, coarse coagulates or by reactions of growing microgels with terminated or with other growing microgels and formation of insoluble agglomerates or aggregates.

As a consequence of the self-emulsifying property of EUP, the ratio EUP/comonomer in the reaction mixture not only determines the composition of the microgels but is also an important factor for their yield. The product profiles of microgels, prepared by ECP of EUP/styrene (S) (Fig. 17) and of EUP/methylmethacrylate (MMA) (Fig. 18) using a water-soluble initiator, show that an exclusive formation of microgels is limited by the EUP/comonomer ratio and the W/M-ratio. Above a certain EUP/comonomer ratio, microemulsions are formed, and if the W/M-ratio is sufficiently high, microgels are the only reaction product. With high EUP/comonomer ratios, besides microgels insoluble copolymers are obtained. Their formation may be explained by reactions between microgel particles after longer reaction times. With low EUP/comonomer ratios, normal emulsion are formed containing both micelles and monomer droplets. In this case, besides microgels the formation of a macrogel is observed. Its formation may be explained by the reaction between polymerized monomer droplets.

In non-crosslinking ECP, monomers are supplied to the growing polymer species by diffusion of monomer from droplets. In crosslinking ECP, however, the gel effect increases the copolymerization rate in the droplets as well as in the growing microgel particles. As the diffusion rate of lipophilic monomers in the aqueous phase is lower than the copolymerization rate, monomer droplets may

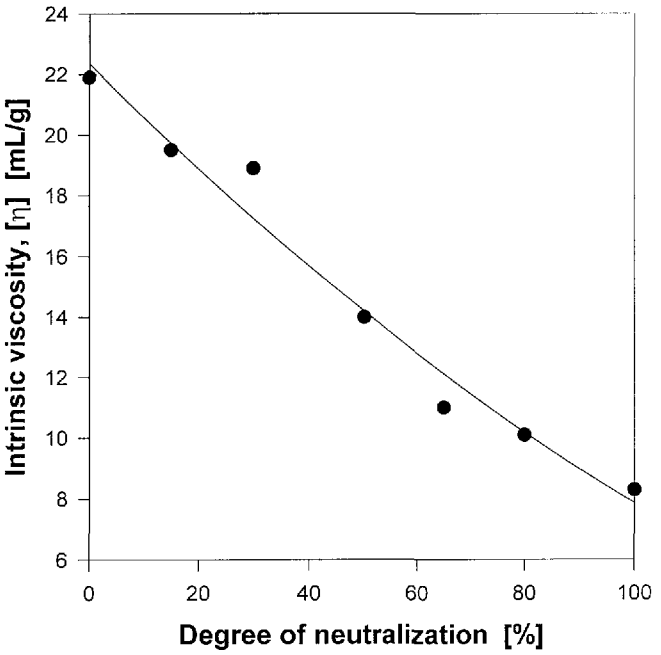


Fig. 19. Influence of the degree of neutralization of the ECP on [η] of microgels [116]. EUP(MA+HD), $\overline{M}_n$ 2100, c/t 77/23, EUP/S 2/3, W/M 5, external emulsifier poly(oxymethylene octylphenyl ether).

be polymerized, despite their much smaller surface area available for entering of radicals from the aqueous phase.

The window in the product profile of the ECP, where microgels are exclusively formed, also comprises the compositions of the reaction mixture in which microemulsions are formed.

The [η] of microgel solutions decreases with increasing degree of neutralization of the carboxyl acid groups of the EUP (Fig. 19) because the emulsifier concentration increases and, accordingly, the micelles or microemulsion droplets become smaller. In this case an external emulsifier poly(oxymethylene) octylphenyl ether was added to insure complete solubilization over the whole range of neutralization.

In order to prevent the formation of macrogels due to the polymerization of monomer droplets and to the reaction between them, the degree of neutralization should be close to 100 %, i.e. the pH of the emulsion should be in the range of complete neutralization which is about pH 8, (Fig. 20). Then a droplet-free microemulsion exists and a sufficiently high EUP fraction protects the growing microgels by electrostatic repulsion from reacting with each other. At a high pH the yield of microgels decreases probably due to agglomeration and degradation of the EUP but the composition of the microgels remains constant.

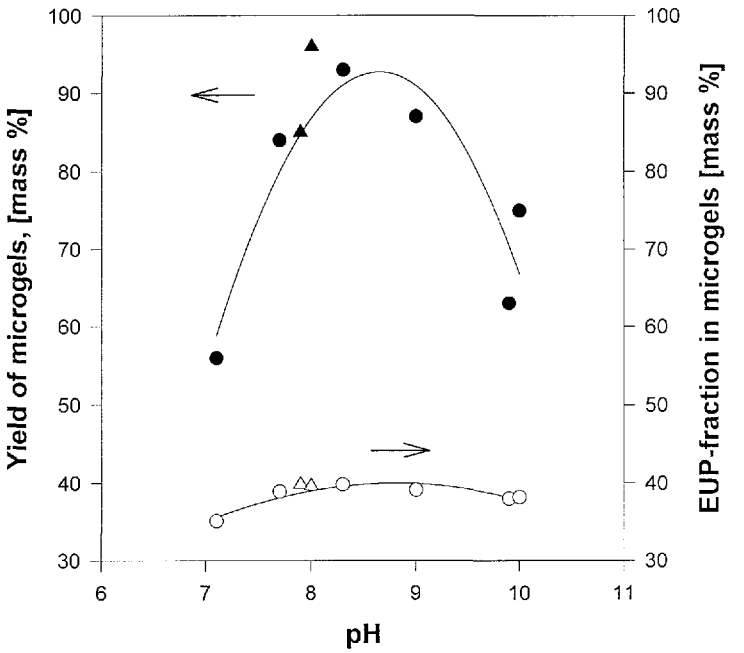


Fig. 20. Dependence of the yield and composition of microgels on pH of the emulsified reaction mixture [116]. (EUP/S: black and white circles 0.33, black and white triangles 1.5; other data see Figure 19).

5.2.1
Molar Mass and Diameter of Microgels

As the EUP is an emulsifier, an increase of the EUP/comonomer ratio not only causes an increase of the number of micelles and microemulsion droplets respectively but also of the number of microgels and, correspondingly, a decrease of their molar mass [110, 126] and their diameter [127]

Because the presence of an electrolyte increases the dimensions of micelles and microemulsion droplets [115], it may be expected that in presence of ions the size of microgels is also increased. This expectation could be confirmed: external electrolyte increases $\overline{M}_w$ (Fig. 21) as well as $\bar{d}_z$ and $[\eta]$ (Fig. 22) up to the limit of the emulsion stability. Therefore, the addition of an external electrolyte to the reaction mixture for the ECP of EUP and comonomers is a means to vary the molar mass, the diameter and the intrinsic viscosity of microgels from EUP and comonomers deliberately.

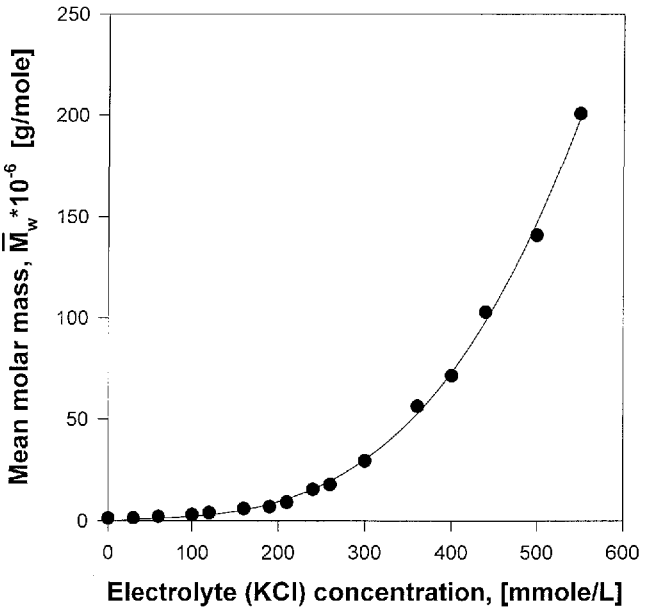


Fig. 21. Influence of an external electrolyte (KCl) on $\overline{M}_w$ (dioxane) [122]. EUP(MA+HD), $\overline{M}_n$ 1290. c/t 77/23, EUP/S 4, W/M 25, pH 7.5.

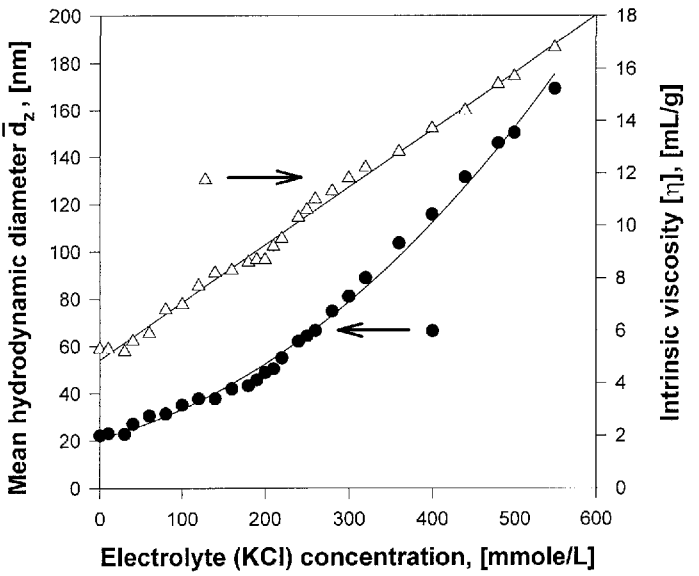


Fig. 22. Influence of the electrolyte concentration (KCl) on $\overline{d}_z$ and [η] (dioxane) [122], (reaction parameters as in Figure 21).

5.2.2
Viscosity

The [η] of microgels increases slightly with the concentration of an external electrolyte (Fig. 23). Probably a slope of $[\eta] / \overline{M}_w > 0$ is caused by the presence of the electrolyte which decreases the density of these microgels.

If persulfate is used as an initiator, its decomposition and the reactions of the radicals formed are rather complex [118]. Sulfate radicals and hydroxyl radicals are formed and may add to the unsaturated acid units of the EUP or are introduced into the surface of microgels, thus making them more hydrophilic and influencing their surface properties [81]. Moreover, persulfate radicals also react with the carboylic acid groups of the EUP, as had been shown by the accelerated decomposition of this initiator in presence of EUP [128]. Contrary to these disadvantages, the radical fragments of AIBN do not change essentially the chemical character of the growing chains and of the microgel surface and therefore are more suitable for the initiation of ECP.

Compared with persulfates, the solubility of AIBN in water is very low (Fig. 24). At the usual reaction temperature of the ECP (70 °C) only about 2 mg of this initiator dissolves in 1 l of water. This means that, irrespective of the distribution ratio in both phases, most of the AIBN in the usually applied concentration range (about 1–6 g/l) is dissolved in the non-aqueous phase. Conse-

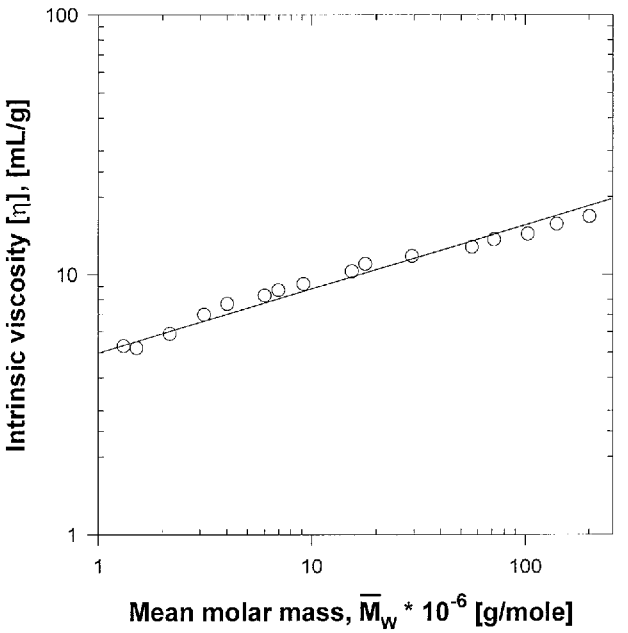


Fig. 23. Relation between [η] and $\overline{M}_w$ (dioxane) [122](reaction parameters as in Fig. 21).

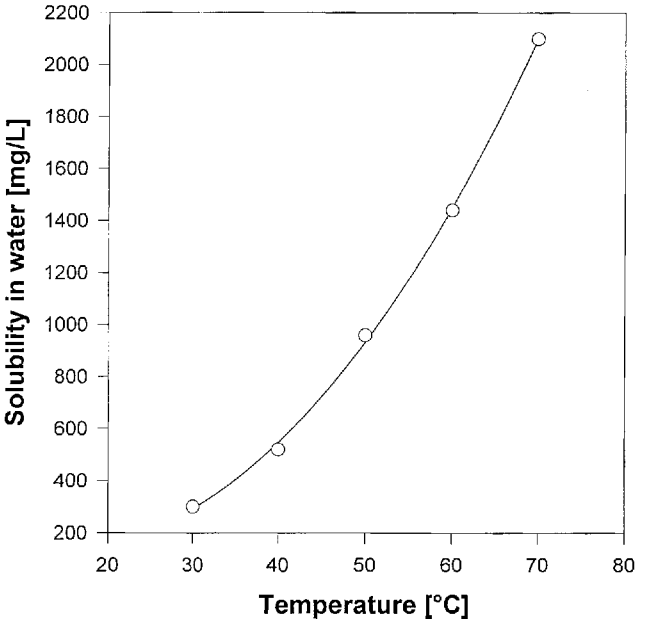


Fig. 24. Dependency of the solubility of AIBN in water on the temperature [128].

quently, contrary to earlier conclusions [129], AIBN, due to its low solubility in water and its higher decay rate in presence of EUP [128], decomposes predominantly in the lipophilic phase of an aqueous emulsion. Therefore, ECP is initiated in the micelles or in microemulsion droplets and not in the aqueous phase.

Because the copolymerization of the components of micelles is very rapid, the microgel particles scarcely grow by intermicellar diffusion of the comonomers or by diffusion from the microemulsion droplets. This has been confirmed by the microgel composition [112] which remains constant over the whole reaction time (Fig. 25), even when using different ratios of EUP/comonomer [113, 116].

A small increase of the molar mass during the copolymerization [115] is explained by an incorporation of not yet initiated micelles or droplets of the microemulsion in the growing microgels or by their aggregation to larger particles.

5.3 Characterization and Properties of Microgels from Self-Emulsifying Unsaturated Polyesters and Comonomers

The molar mass of microgels obtained by ECP of EUP and comonomers ranges from below 10^6 to more than 10^7. Similar to the decrease of the particle size with increasing concentration of other emulsifiers, an increase of the EUP-fraction in the monomer mixture decreases the $\overline{M}_w$ of the microgels (Fig. 26).

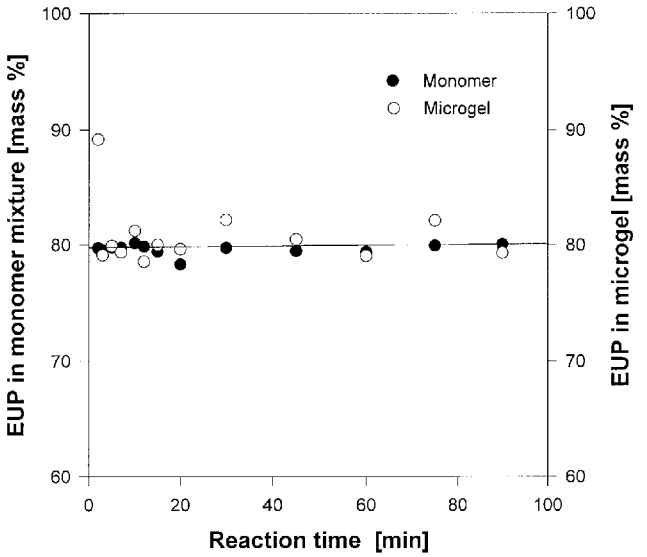


Fig. 25. Composition of microgels and of the reaction mixture (EUP/S) in the course of the micro-ECP [112]. EUP(MA+HD), $\overline{M}_n$ 1290, c/t 77/23, W/M 25, KCl 200 mmole/L, AIBN.

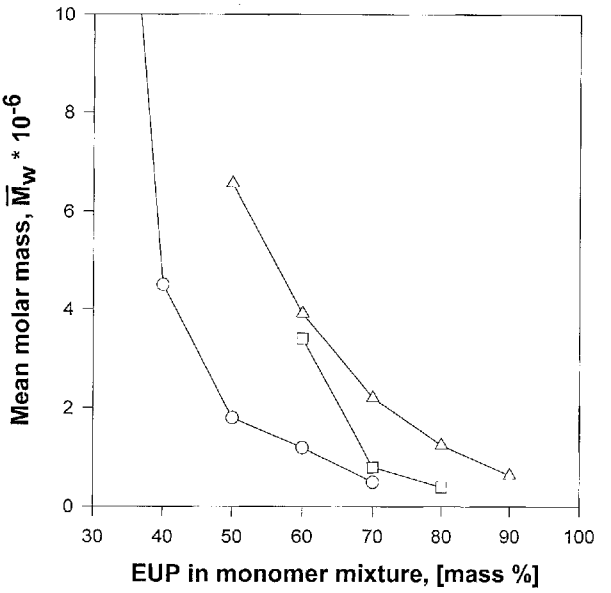


Fig. 26. Relation between the EUP-content in the reaction mixture and $\overline{M}_w$ of microgels. EUP(MA+HD), M_n 1300, c/t 75/25, AIBN [115]. EUP(MA+PA+HD), $\overline{M}_n$ 1330, c/t 71/29, EUP/DVB, W/M 20 [130]. EUP(MA+PD+HD), $\overline{M}_n$ 1330, c/t 71/29, EUP/EDMA, W/M 30 [130].

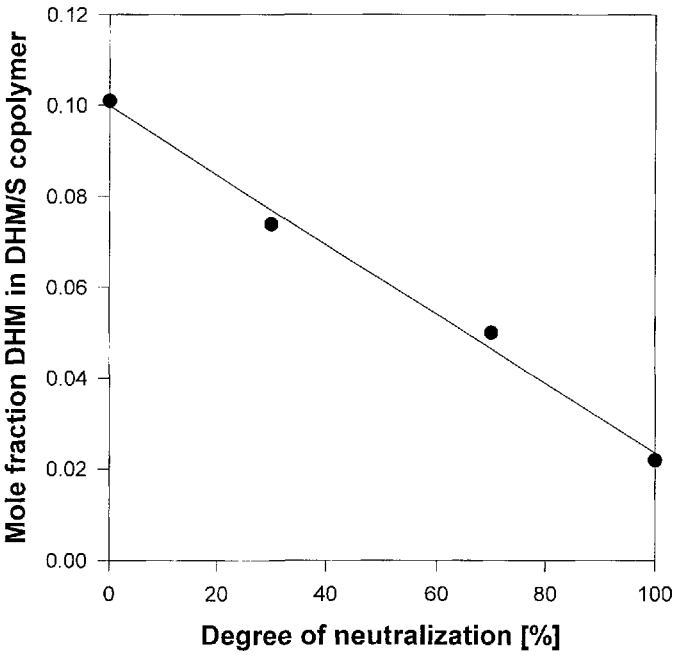


Fig. 27. Relation between the degree of neutralization and the mole fraction of dodecyl hydrogen maleate (DHM) in the copolymerization with S [131]. (DHM/S in reaction mixture 0.133).

Viscosity, dispersion stability and reactivity of microgels from EUP and comonomers are influenced by the location, concentration and dissociation of terminal carboxyl acid groups. In the dissociated state, terminal acid groups deactivate the double bonds of the neighboring unsaturated terminal units [116]. This deactivation is very obvious in the copolymerization of half-esters of maleic (Fig. 27) and fumaric acids with styrene. As Fig. 27 shows, the incorporation of dodecyl hydrogen maleate (DHM) in the copolymerization with S is rather low and strongly decreases further with increasing neutralization of the acid groups. As a consequence, short chains of terminal units of EUP-molecules remain unreacted at the surface of the microgel particles. The presence of these unsaturated terminal units of the EUP could also be confirmed by the formation of pyrazolin dicarbonic acid units with CH_2N_2 [132]. However, due to hydrolysis, even on complete neutralization still enough reactive terminal ester units are available at the surface of microgels for copolymerization. It may be assumed, therefore, that the compactness of the microgel particles is not essentially decreased by the deactivation of terminal unsaturation.

5.3.1
Viscosity and Hydrodynamic Diameter

An interesting feature of microgels is that, unlike crosslinked polymers, they are soluble in suitable solvents and can therefore be characterized by the viscosity of their solution. As compared with linear macromolecules of the same molar mass and composition, microgels have a rather compact structure. If microgels behave like rigid solid spheres, according to the Einstein law the intrinsic viscosity, [η] should only depend on the density of the particles and not on their molar mass. However, even with a uniform density of the microgel particle throughout its volume, [η] may depend on the thermodynamic quality of the solvent and on the crosslink density. Provided the same solvent is used and the composition of the microgels is the same, their crosslink density may be related to their [η]. In this case viscosity measurements can be used for determining the crosslink density of a microgel network.

As may be seen in Figs. 28 and 29, values for [η] of various microgels from UP and S resp. 1,4-DVB and EDMA are only as low as about 4–8 ml/g and depend little on the molar mass over a range of about 0.5×10^6 to 40×10^6 g/mol. As compared with these values, the [η] of linear polystyrene for the same range of molar

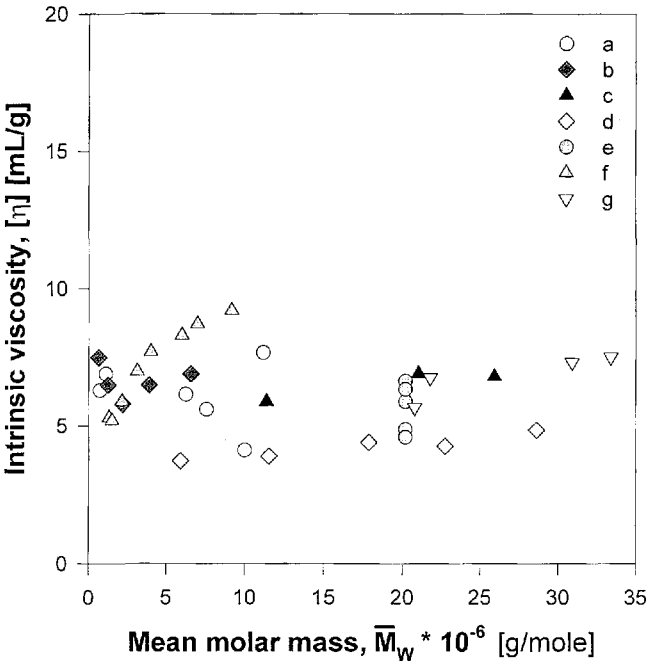


Fig. 28. Relation between [η] (dioxane) and $\overline{M}_w$ of microgels from various EUP and bifunctional comonomers.a): [136], b): [115], c),d),e): [116], f): [122], g): [132])

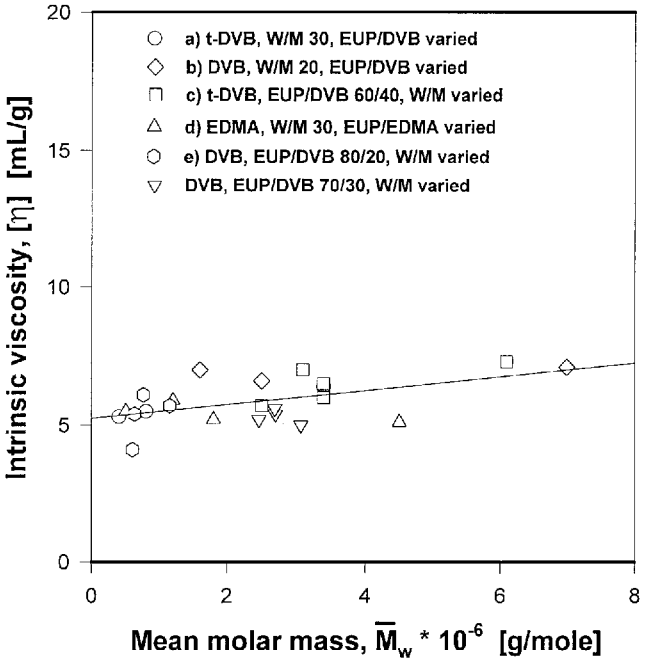


Fig. 29. Relation between [η] (dioxane) and $\overline{M}_w$ of microgels from various EUP and tetrafunctional comonomers a) EUP(MA+PA+HD), $\overline{M}_n$ 1330, c/t 71/29, W/M 30, $K_2S_2O_8$ [130]. b) EUP(Ma+HD), $\overline{M}_n$ 1300, c/t 75/25 , W/M 20, AIBN [115]. c) EUP(MA+PA+HD), see a), $K_2S_2O_8$ [130]. d) EUP(MA+PA+HD), W/M 30, see c) [130]. e) and f) EUP(MA+PA+HD), $\overline{M}_n$ 1270, c/t 84/16, W/M varied, $K_2S_2O_8$ [121].

mass would extend from about 160 to 3900 ml/g, calculated by $[\eta] = K \times \overline{M}^a$ with $K = 11 \times 10^{-3}$ and a = 0.73. The scattering of the points in Fig. 28 is due to experimental variations, such as UP/S ratio, molar mass of the UP, serum ratio and concentration of the initiator.

In agreement with the decrease of $\overline{M}_w$ of microgels on increasing the amount of EUP in the monomer mixture (Fig. 26), their mean particle diameter likewise decreases (Fig. 30). With the molar mass of microgels also their diameter increases (Fig. 31). However, a 20-fold increase of the $\overline{M}_w$ corresponds to only less than a 3-fold increase of d_z. These results illustrate results that microgels from EUP are rather compact globular particles with intrinsic viscosities closely approaching that of hard spheres.

As to the homogeneity of microgels, their composition and their structure has to be considered. In an aqueous alkaline solution a stepwise degradation of microgels by hydrolysis is possible [133], by which especially the unreacted terminal EUP-units are removed [115]. The degradation rate increases with the EUP-fraction incorporated in the microgel.

Because the composition of microgels prepared by micro-ECP of EUP and styrene with AIBN as initiator remains constant and irrespective of the reaction

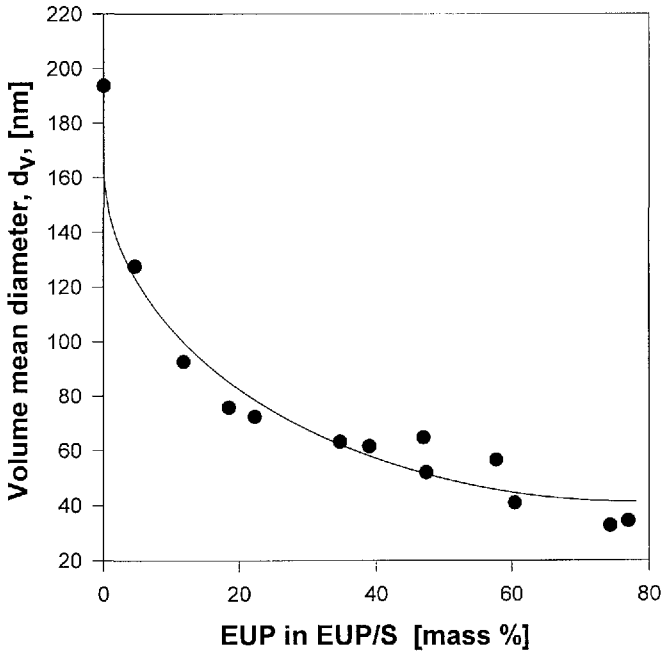


Fig. 30. Dependency of $\bar{d}_v$ of microgels on the EUP-content of the monomer mixture [127]. EUP(MA+HD), $\overline{M}_n$ 2700, c/t 80/20, W/M 15, $K_2S_2O_8$, external emulsifier poly(oxymethylene octylphenyl ether).

time, it is practically the same as that of the monomer mixture [112, 113, 116], it follows that these microgels have a homogeneous composition. This means that during the reaction diffusion of monomers from not yet initiated micelles to growing particles is negligible. Otherwise, a change of the composition would be expected because the rates of diffusion of EUP and styrene certainly are very different. However, because the reactivity ratios of the copolymerizing components differ significantly, a structural inhomogeneity is possible, especially with high amounts of the bifunctional comonomer or with crosslinking comonomers such as DVB.

The parameters which influence the particle size of microgels have been studied during self-emulsifying, seeded emulsion copolymerization of an unsaturated polyester and butyl acrylate [134].

5.3.2 *Reactive Groups*

The reactivity of microgels resides in terminal carboxyl acid groups and in residual unsaturated dicarboxylic acid groups of the EUP-component. Due to sterical hindrance, presence of less reactive maleic acid units and deactivation of termi-

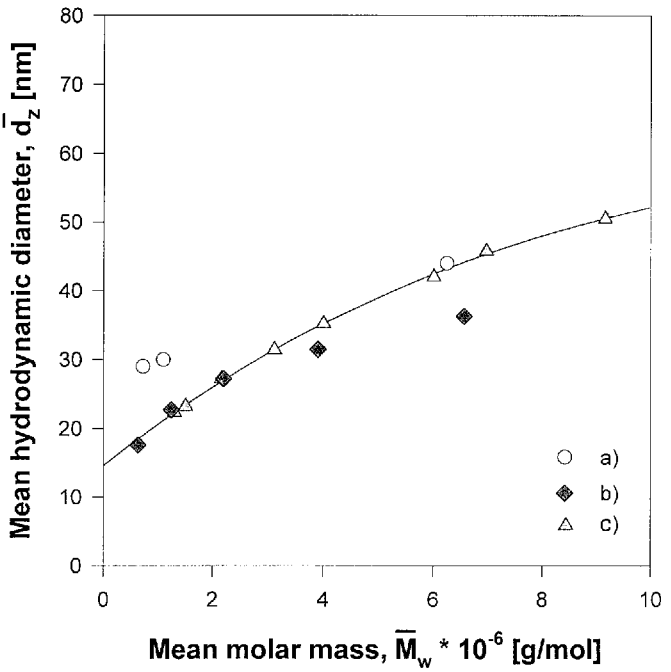


Fig. 31. Relation between $\bar{d}_z$ of microgels and their $\overline{M}_w$. a): [136], b): [115], c): [122].

nal carboxylate groups, a relative large fraction of unsaturated units remains unreacted within and at the surface of microgels (Fig. 32). This residual unsaturation increases with the EUP-fraction in the microgels because the crosslink density increases and therefore the mobility of reactive chain segments decreases.

Independently of the microgel composition, the fraction of terminal acid groups of the EUP-component determined by conductivity titration is only about 75 mol % of the total amount of acid groups incorporated in the microgels by polymerization (Fig. 32). This means that the residual 25 mol % acid groups are located within the microgel particles and are not easily accessible by ions. It may be assumed that these interior acid groups have been in the free acid state during the copolymerization due to hydrolysis of carboxylate groups.

A possible reason for the inaccessibility of a part of the acid groups could be the crosslink density which depends on the composition of the microgels. However, because the number of titratable acid groups does not depend on the composition and, therefore, on the crosslink density of the microgels, it must be concluded that electrostatic forces prevent ions from entering the microgel particles.

Solutions of microgels from EUP and bifunctional comonomers are rather stable over weeks and months. However, on exposing freeze-dried samples of microgels from ECP of EUP and S to O_2 or N_2, insoluble fractions are formed which increase with exposure time and temperature. As insolubilization is prevented in

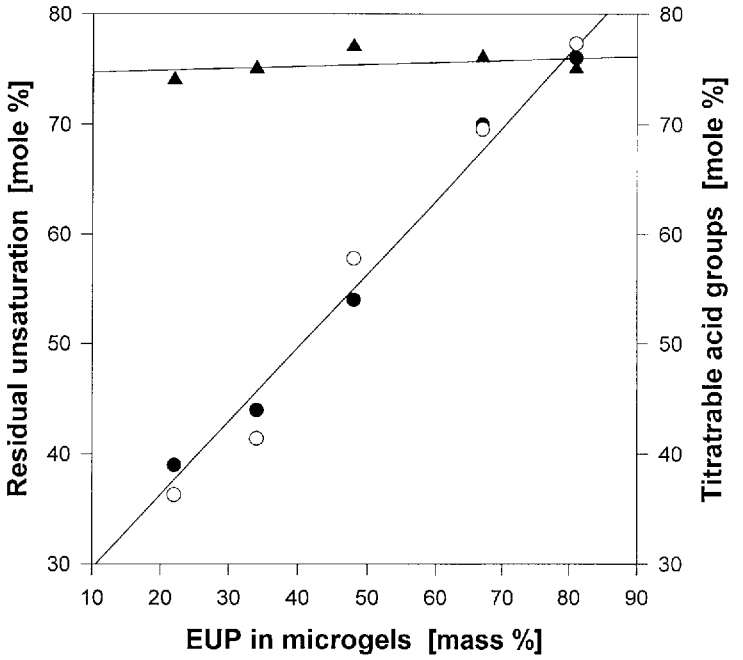


Fig. 32. Relation between the residual unsaturation ( ) IR-spectroscopy, (○) hydrolytic degradation resp. the titratable acid groups of microgels ( ) and their EUP-content [132]. EUP(MA+HD), $\overline{M}_n$ 1640, c/t 67/33, EUP/S varied, W/M 20.

presence of radical inhibitors, it is probably caused by reactions between these particles in their non-swollen state via pendent unreacted groups of the EUP [116].

On repeated freeze-drying of microgels with EUP-components an irreversible formation of an insoluble aggregate was observed [135]. It was supposed that this aggregation is due to radical reactions between adjacent microgel particles. The radicals are possibly formed by a mechanical rupture of chains due to stresses within the particles caused by freezing.

5.3.3
Rheological Properties of EUP/Comonomer-Microgels

Rheological properties of microgels composed of EUP (MA and HD) and S, EDMA, resp. DVB have been measured in 2-ethoxyethylacetate [136]. Below concentrations of 40 mass %, very low viscosities and an almost Newtonian flow have been observed (Fig. 33). At higher concentrations, shear thickening is observed. Accordingly, these microgels are rather compact particles that intereact very little with each other and are not deformed by shearing up to high concentrations, where the close packing causes rheopexy. The compactness of EUP/S-microgels has been also confirmed by 2H-NMR spectroscopy using selectively deuterated components [137].

		Polymer Concentration [mass %]						
Monomer	UP/M	40	35	30	25	20	15	
Styrene	1.5	1	9					
Styrene	0.67	2						
Styrene	1	3	8	13+18				
Styrene	1		6	12+17	15+20	21	22	
Diacrylate	0.67		7	11+16	14+19			
Polystyrene					4	5	10	
Ethylglycol acetate	Solvent							23

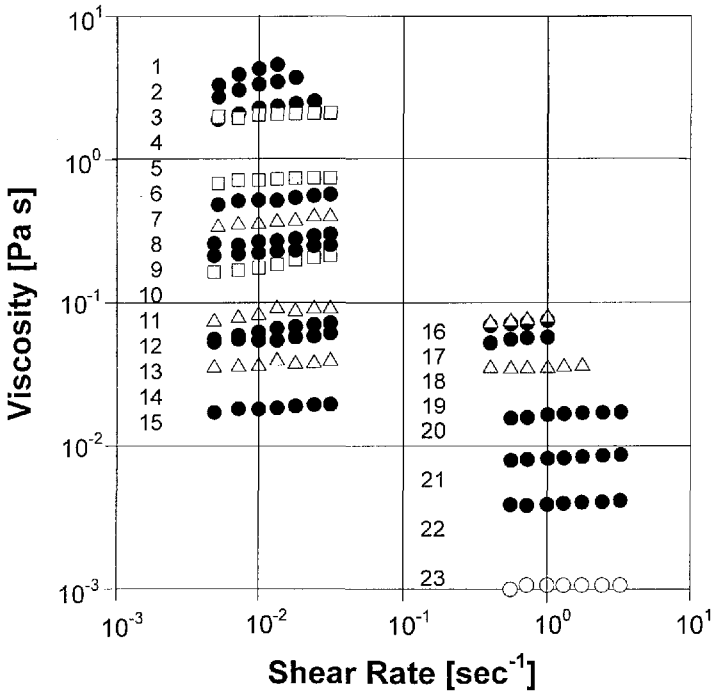


Fig. 33. Dependence of viscosity on the shear rate of microgel solutions in $C_2H_5OC_2H_4OCOCCH_3$. EUP(MA+HD), c/t 70/30, EUP/S and EUP/EDMA(D), AIBN, P.-S. polystyrene [136].

6 Microgel Formation in Solution by Free-Radical Crosslinking Copolymerization

6.1 Theoretical Considerations

Several theories of network formation have been developed in the past half century, including statistic [50, 64–66, 138–144] and kinetic ones [100, 145–153], and

simulation of network formation in a n-dimensional space, such as the percolation theory [154–156]. However, up to now, no exact theory of network formation for radical crosslinking copolymerization (RCC) exists that takes into account heterogeneities and microgel formation due to an extensive cyclization and multiple crosslinking. This deficiency is explained by the complicated mechanism of these reactions. If long-range correlations such as cyclization and multiple crosslinking with resulting heterogeneities are neglected, kinetic approaches may successfully solve the complex mechanism of RCC. Deviations observed in real systems are then useful for understanding the reasons for the non-ideal behavior.

Radical polymerizations have three important reaction steps in common: chain initiation, chain propagation, and chain termination. For the termination of chain radicals several mechanisms are possible. Since the lifetime of a radical is usually less than 1 s, radicals are continuously generated and terminated. Each propagating radical can add a finite number of monomers between its initiation and termination. If a divinyl monomer is in the monomer mixture, the reaction kinetics changes drastically. In this case, a dead polymer chain may grow again as a macroradical, when its pendant vinyl groups react with radicals, and the size of the macromolecule increases until it extends over the whole available volume.

RCC involves at least two types of vinyl groups which have different reactivities [100], those of the monomers and those of pendant vinyl groups. Accordingly, the homopolymerization of divinyl monomers can be considered as a special case of copolymerization, in which the second vinyl group of the divinyl monomer changes its reactivity after the first vinyl group has polymerized. During RCC the pendant vinyl groups thus formed can still react or remain pendant. Understanding the behavior of pendant vinyl groups is a key for explaining the formation of microgels.

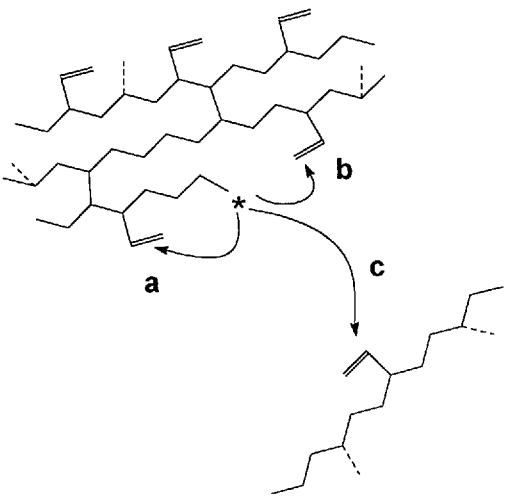


Fig. 34. Schematic picture of cyclization (a), multiple crosslinking (b), and crosslinking (c) in radical crosslinking copolymerization.

Two possible reactions of a pendant vinyl group may be distinguished, shown schematically in Fig. 34:

- intramolecular crosslinking (a and b),
- intermolecular crosslinking (c).

Intramolecular crosslinking occurs between pendant vinyls and radical centers located on the same macromolecule and results in the formation of cyclic chains and multiple crosslinks [157]. A cyclic chain is formed if both, the pendant vinyl group and the radical center are located on the same kinetic chain (a); otherwise a multiple crosslink (b) is formed. Cyclic chains can be of a short-range type, e.g. loops within a monomer, or of a long-range type, i.e. between radical centers and pendant vinyl groups located at different distances in the same kinetic chain [100]. Chain cycles and multiple crosslinks do not contribute to the growth of the macromolecule and have no influence on the onset of macrogelation but cause the macromolecules to contract and thus reduce their size. The contraction of the macromolecules by intramolecular crosslinking also reduces the reactivity of pendant vinyl groups by steric hindrance. It should be mentioned that cyclization and multiple crosslinking were recently re-defined as primary and secondary cyclization [147], or as intramolecular cyclization and intramolecular crosslinking, respectively [30]. In the present review, the classical definitions will be used.

Intermolecular crosslinking between pendant vinyl groups and radical centers located on different macromolecules produce crosslinks that are responsible for the aggregation of macromolecules, which leads to the formation of a macrogel. It must be remembered that both normal and multiple crosslinks may contribute to the rubber elasticity of a network, whereas small cycles are wasted links.

The divinyl monomers can thus be found in macromolecules as units which bear pendant vinyl groups or which are involved in cycles, crosslinks or multiple crosslinks. Since the number of crosslinks necessary for the onset of macrogelation is very low [64], pendant vinyl groups in RCC are mainly consumed in cycles and multiple crosslinks. Therefore, the reaction rate of pendant vinyl groups is a very sensitive indicator for the formation of cycles and multiple crosslinks in finite species [100, 147, 157–160].

The conversion of pendant vinyl groups, x_3, may be defined as the fraction of divinyl monomer units with both vinyl groups reacted

$$x_3 = \frac{\text{number of divinyl monomer units in the polymer with both vinyl groups reacted}}{\text{total number of divinyl monomer units in the polymer}} \qquad (6)$$

x_3 is zero for linear chains bearing pendant vinyl groups only, and unity for chains carrying only divinyl monomer units with both vinyl groups reacted. Assuming no cyclization, every divinyl monomer unit in the polymer should initially bear a pendant vinyl group, i.e., $\lim_{x \to 0} x_3 = 0$, where x is the monomer conversion. Since crosslinking and multiple crosslinking are second order reactions,

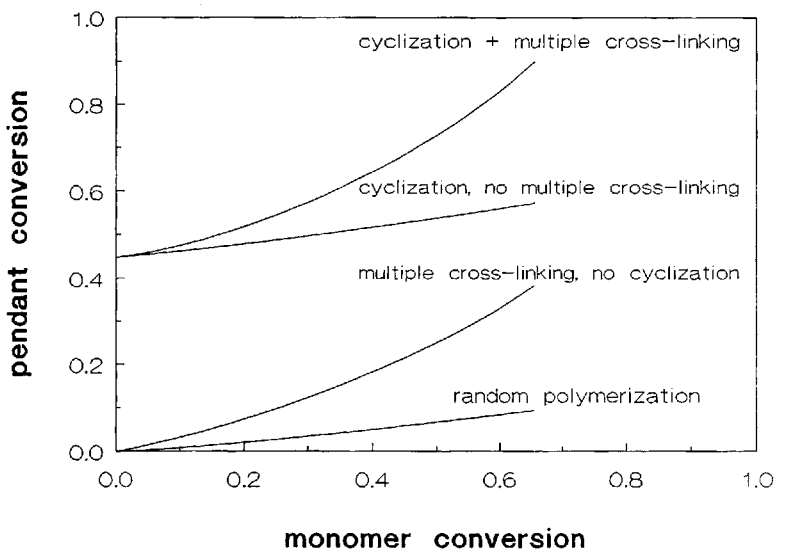


Fig. 35. Graphical representation of variation of the conversion of pendant vinyl groups x_3 with the monomer conversion x for various types of intramolecular reactions [157].

deviation from zero indicates the cyclization. Thus, the initial rate of cyclization can be calculated by plotting the experimentally determined conversion x_3 of pendant groups vs the monomer conversion x and extrapolation to zero monomer conversion. Moreover, the conversion rate of pendant vinyl groups is a measure of the extent of multiple crosslinking [157]. The greater the slope of the curve x_3 vs x curve, the larger is the number of multiple crosslinks formed per crosslink. Therefore, multiple crosslinking is reflected in a greater decrease of polymer unsaturation than without it. Figure 35 shows schematically the variation of the conversion of pendant vinyl x_3 with monomer conversion x for various types of intramolecular reactions.

6.2 Experimental Evidences of Intramolecular Crosslinking

Investigations of intramolecular crosslinking in RCC are found in the literature from as early as 1935. Staudinger and Husemann could isolate a soluble polymer by polymerizing DVB alone in very dilute solutions [4]. Walling observed that the actual gel point in the bulk polymerization of EDMA exceeds that predicted by the classical theory of gelation by more than two orders of magnitude (2.9% vs 0.022% in terms of critical conversion) [161]. This author stated that "the growing chain undergoes so many crosslinking reactions within itself that its ability to swell is reduced" [161]. Zimm et al. observed that $[\eta]$ of branched DVB/S copolymers depends only a little on the molar mass [162]. They found an exponent a = 0.25 of the Mark-Houwink equation which is between the value for

rigid spheres (a = 0) and that of an unperturbed Gaussian chain (a = 0.50). Storey observed that in 1,4-DVB/S copolymerization the critical conversion passes through a minimum at the gel point when the content of 1,4-DVB is increased [32]. He explained this unusual gelation behavior with macrogelation by an accumulation of microgels that have a high crosslinker content. Malinsky et al. observed that in 1,4-DVB/S copolymerization the fraction of pendant vinyl groups is lower at low conversions than calculated, whereas at high conversions the copolymers contain a large excess of these groups [33]. These authors explained their results by cyclization and reduced mobility of chain segments. Immobilization dominates at high conversions and reduces the reactivity of pendant vinyl groups. In studying the polymerization of pure divinyl monomers, Kast and Funke found that at no time during the polymerization in solution could linear or branched polymers be isolated, but only intramolecularly crosslinked polymers of high crosslink density [55]. They concluded that at high crosslinker contents a macroscopic gel forms via reaction between the functional groups of the microgel particles after enough microgel particles have been formed to fill the reaction volume. Galina and Rupicz found that in copolymerization of EDMA/S in benzene only a small fraction of EDMA units are involved in intermolecular crosslinks [163]. They concluded that cyclization and multiple crosslinking are the most important features of this polymerization. Dusek et al. emphasized the importance of cyclization and reduced reactivity of pendant vinyl groups in RCC and proposed a mechanism of macrogelation via microgels [56]. Cyclization and the reduced reactivity of pendant vinyl groups in RCC during the gel formation were also pointed out by many other researchers [28, 38, 164–186]. A consequence of cyclization and multiple crosslinking is the appearance of multiple glass transitions [187, 188], the existence of trapped radicals [189–192] and residual unsaturation in the final networks [193].

It was also shown that in RCC intra- and intermolecular crosslinking enhance the Trommsdorf [194] or gel effect significantly. The autoacceleration of the polymerization rate begins shortly after the start of the polymerization [160, 195–197]. The termination reactions are controlled by the rate of translational diffusion of chain segments and the radical chains. However, after the aggregation of the primary particles via multiple crosslinks, free radicals bound to aggregates should have extremely small diffusion coefficients. For such species, it is easy to imagine that they are immobile (trapped) in the time-scale of the kinetic events. Under these conditions, bimolecular termination in the particles can occur only by diffusion of two free-radical chain ends toward each other as a result of their propagational growth ("reaction diffusion" or "residual termination" mechanism) [198–200]. Indeed, in case of bulk polymerizations of divinyl monomers, the ratio of rate constants termination/propagation was found to be constant [201, 202]. On the other hand, it was also reported that the primary chain length, i.e. the chain length of polymers close to zero conversion or when connections between unsaturated groups in bisunsaturated monomer units are severed, increases with increasing crosslinker content in the monomer mixture [197, 203–206]. This unusual behavior was explained by cyclization, which decreases the mobility of segments and suppresses the diffusion-controlled termination due to steric reasons [204].

Tobita and Hamielec used the dependency between pendant vinyl conversion x_3 on the monomer conversion x of several systems to calculate the fraction of divinyl monomer units involved in formation of cycles [158, 207]. They showed that in copolymerization of *N,N'*-methylene bisacrylamide and acrylamide in water (56.6 g comonomers/l) at least 80% of the pendant acryl groups are consumed by cyclization reactions. For the same system they also showed that the consumption of pendant acryl groups by multiple crosslinking is much greater than that by normal crosslinking. Assuming constant rates for intramolecular crosslinking, they calculated that, on average, 10^3 multiple crosslinks form per intermolecular crosslink [158]. Landin and Macosko [147], and more recently Dotson et al. [205] attempted to measure conversions of the pendant double bond in EDMA/MMA copolymers by NMR. They showed that both ^1H and ^{13}C NMR techniques result in negative values for the conversion of pendant methacrylic groups due to the decreased mobility of protons in intramolecularly crosslinked molecules. By using an analytical titration method, Okay et al. found that in dilute solutions almost half of the pendant double bonds of EDMA units are consumed by cyclization [197]. More recently, Dusek and coworkers studied the RCC of styrene with bismaleimide, *p*-maleimide, *p*-maleimidobenzoic anhydride, or with mixtures of *p*-maleimidobenzoic anhydride and methyl *p*-maleimidobezoate [208]. Their results also demonstrate the important role of cyclization in the early stage of crosslinking copolymerization and steric hindrance of pendant unsaturated groups at higher conversions.

Due to the sensitive dependence of the gel point on the reactivity of pendant vinyl groups for intermolecular links, it is possible to estimate the reactivity ratio of pendant to monomeric vinyl groups from experimental data. In 1,4-DVB polymerization in toluene the average pendant reactivity was found to be 2–3 orders of magnitude lower than the monomeric vinyl reactivity [209]. Lower pendant vinyl group reactivities were also calculated in EDMA/MMA and *N,N'*-methylene bisacrylamide/acrylamide copolymerization in dilute solutions [206, 210]. The decrease in pendant reactivity indicates a thermodynamic or steric excluded volume effect [30, 31]. It should be noted that both, the number of cycles and multiple crosslinks as well as the reactivity of pendant vinyl groups are functions of monomer conversion. It may be expected that no multiple crosslinks exist at zero monomer conversion and that their number increases as the reaction proceeds because multiple crosslinking becomes more probable if the macromolecules are larger. The opposite behavior can be expected for the cycle formation. On the other hand, increasing the number of multiple crosslinks during the reaction would cause a decrease of reactivity of the pendant groups because they are increasingly shielded [211].

It is obvious that intramolecular crosslinking is always observed in radical polymerization of divinyl monomers or divinyl/vinyl comonomers. Thus the experimental results clearly show that the prediction of ring-free theories fail. At the beginning of the reaction, the polymer radicals in a monomer/solvent mixture are rather isolated from each other. Hence the local concentration of pendant vinyl groups inside a macroradical coil is much higher than their overall concentration in the reaction mixture. Consequently, the probability of the radical chain end attacking a pendant vinyl group of its own chain is strongly

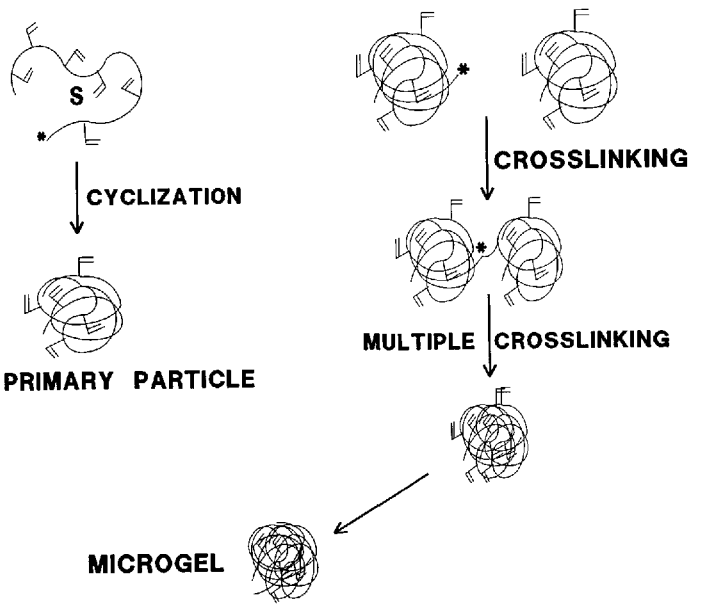


Fig. 36. Schematic representation of microgel formation in RCC.

favored, and in the early stage of RCC chain cycles are predominantly formed leading a to decreased size of coils of the same molar mass. Since every cycle reduces the coil dimensions as well as the monomer content inside the coil, the structure of the polymers is rather compact. Such crosslinked polymer coils may be considered as primary particles, analogous to the primary molecules as intermediates in the classical theories of gel formation [64] (Fig. 36). With increasing conversion the concentration of these primary particles increases and so does the opportunity to be added to a pendant vinyl group at the surface of some other particles. This intermolecular crosslinking leads to polymer aggregates. Since the concentration of pendant vinyl groups in a particle increases rapidly after the formation of each crosslink (Fig. 36), a number of multiple crosslinks is expected to occur after each single crosslink which results in a further reduction of the size of these aggregates. Accordingly, microgels isolated in solution polymerization may be considered as aggregates of intramolecularly crosslinked primary particles formed by multiple crosslinking.

6.3 Microgel Synthesis by Radical Copolymerization

Funke and coworkers extensively studied the conditions for the synthesis of 1,4-DVB microgels in dilute solutions of toluene, using AIBN as initiator [209, 212]. They prepared homologous series of 1,4-DVB microgels by a systematic variation of the polymerization temperature, the monomer and the initiator concen-

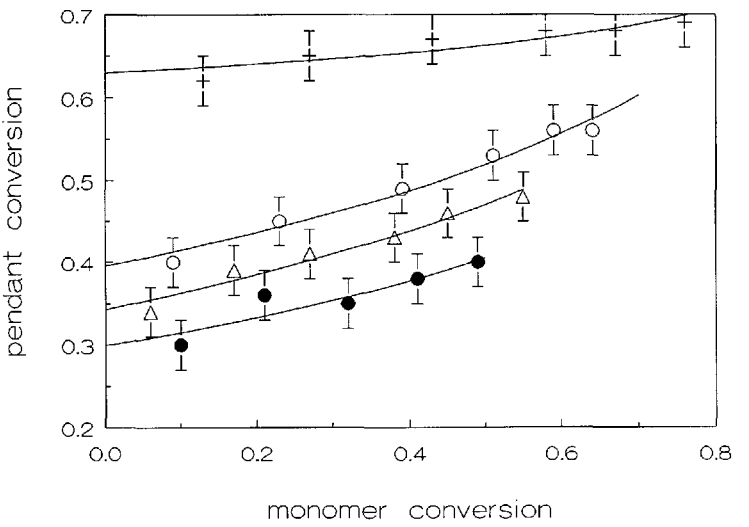
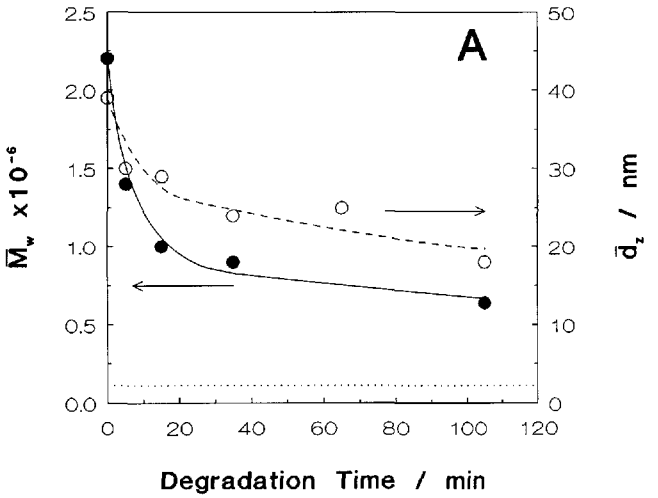


Fig. 37. Conversion of pendant vinyl groups x_3 versus monomer conversion x for different degrees of initial dilution in RCC of 1,4-DVB. Monomer concentration in toluene are 5 ( ), 2 (△), 1 (○), and 0.5 g/100 mL (+). Initiator (AIBN) concentration = 8×10^{-3} M; temperature = 70 °C. [Reprinted with permission from Ref. 209, Copyright 1995, American Chemical Society].

tration. Figure 37 shows representative plots of the conversion of pendant vinyl groups x_3 vs the monomer conversion x for different initial monomer concentrations. Extrapolated values of the conversion of pendant vinyl group to zero monomer conversion indicate that, as the initial monomer concentration decreases from 5 to 0.5 g/100 ml the fraction of microgel units in cycles increases from 0.30 to 0.63 (Fig. 37). An increase of the initiator concentration also increased the fraction of units in cycles. This result may be explained by a more efficient consumption of pendant vinyl groups by cyclization in small particles than in large particles for sterical reasons [212]. It was also shown that in 1,4-DVB/S copolymerization the fraction of units in the chain cycles is a function of the 1,4-DVB content at low amounts of this crosslinker, but not at crosslinker contents as high as 40 mass %. The experimental results indicated that 30–60% of monomer units in 1,4-DVB microgels are engaged in cycles and that on average 100–800 multiple crosslinks exist per intermolecular crosslink [209]. According to these results, a large number of multiple crosslinks are formed between two primary particles after they are linked together by a single crosslink. This also means that in the final macrogels highly crosslinked regions exist which are stable against degradation to primary particles.

In order to check these results, Lutz et al. degraded polymer samples which had been isolated shortly before macrogelation, by ultrasonic waves [213]. Figure 38A shows the decrease of $\overline{M}_w$ and of the hydrodynamic diameter $\overline{d}_z$, measured by static and dynamic light scattering respectively, on ultrasonic treatment of a polymer of $\overline{M}_w = 2.2\times10^6$. Both $\overline{M}_w$ and $\overline{d}_z$ decrease first abruptly but then



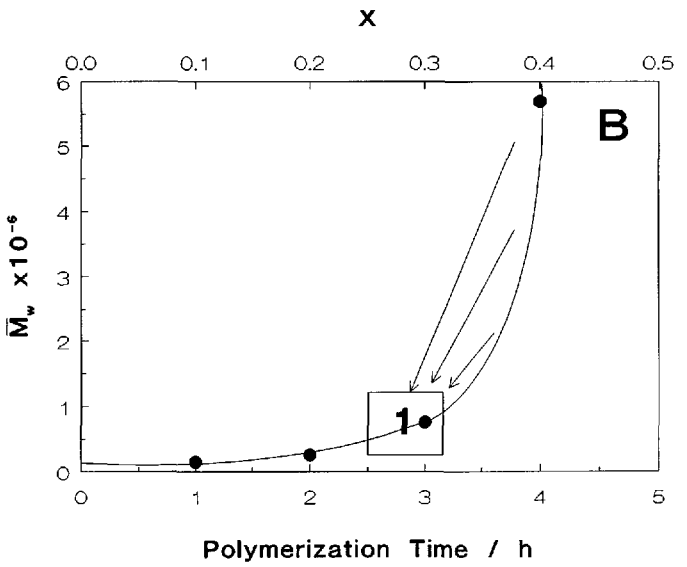


Fig. 38. **A:** Degradation experiments with pregel polymers isolated prior to the onset of macrogelation in 1,4-DVB polymerization [209]: Variation of $\overline{M}_w$ ( ) and d_z (○) with the time of ultrasonic degradation. The polymer sample was prepared at 5 g/100 mL monomer concentration and its initial $\overline{M}_w$ was 2.2×10^6 g/mol. The dotted horizontal line shows $\overline{M}_w$ of zero conversion polymers ("individual microgels"). **B:** Variation of $\overline{M}_w$ with the polymerization time t and monomer conversion x in 1,4-DVB polymerization at 5 g/100 mL monomer concentration. The region 1 in the box represents the limiting $\overline{M}_w$ reached by degradation experiments. [Reprinted with permission from Ref. 209, Copyright 1995, American Chemical Society].

slowly and finally reach a limiting value. This final $\overline{M}_w$ was about 0.64×10^6 g/mol, compared with the molar mass of the primary particles of 0.11×10^6 g/mol (shown as a dotted line in Fig. 38 A) [209]. Under the same experimental conditions, poly(4-methylstyrene) chains could be degraded to a molar mass of 0.08×10^6 [213]. In several experiments with polymers of different molar mass the molar mass decreased down to the region 1 shown in Fig. 38B. These experiments confirm the existence of highly crosslinked regions in microgels due to an extensive multiple crosslinking. Only the large microgel aggregates formed shortly before macrogelation can be degraded.

Chen et al. synthesized microgels by copolymerization of 1,4-DVB and MMA in the presence of a chain transfer agent (CBr_4) [214]. They showed that when the concentration of the chain transfer agent becomes high, the intermolecular crosslinking is depressed and microgels are formed. During the polymerization the structure of the microgels gradually became tight [215] which demonstrates the important role of multiple crosslinks in the formation of microgels.

In order to obtain hydrophilic microgels with sulfo groups, Huang et al. studied the copolymerization of 2-acrylamido-2-methylpropane sulfonic acid and *N,N'*-methylene bisacrylamide in dilute aqueous solutions with potassium persulfate (PPS) as the initiator [216]. By varying the monomer concentration and the crosslinker content of the monomer mixture they obtained reactive microgels with $\overline{M}_w$ up to 25×10^6 g/mol. From the reduced reactivity of sulfo groups in the interior of the microgels, a core-shell structure was assumed with a densely crosslinked core surrounded by a shell of polymerized sulfonic acid monomer. The dimension of this shell varied with its amount in the initial monomer mixture [216].

Microgels can also be synthesized by intramolecular crosslinking of preformed polymers bearing functional groups. Batzilla and Funke prepared linear poly(4-vinylstyrene) (PVS) by anionic polymerization of 1,4-DVB (see next section) and subsequently crosslinked this polymer dissolved in toluene, using AIBN as initiator [217, 218]. They followed the intra- and intermolecular crosslinking reactions by viscosimetry, dynamic and static light scattering and by spectroscopic methods. If only cyclization takes place, the initial $[\eta]$ should decrease during the reaction without any change of the molar mass. An increase in $\overline{M}_w$ is then a sensitive measure for intermolecular crosslinking.

Figure 39A shows, how $[\eta]$ and $\overline{M}_w$ change during crosslinking of PVS of initial molar mass of $\overline{M}_{w,0}$, ranging from 0.3×10^6 to $2{,}4\times10^6$ g/mol. With increasing molar mass, $[\eta]$ decreases first but then increases. This decrease can be explained by a prevailing intramolecular crosslinking, the following increase being determined by intermolecular crosslinks. The minimum of $[\eta]$ indicates the transition from prevailing intramolecular crosslinking to prevailing intermolecular crosslinking. As $\overline{M}_{w,0}$ increases, the minimum of $[\eta]$ becomes more pronounced.

It is well-known that the coil density of macromolecules decreases with increasing molar mass. Due to cyclization this decrease in density becomes less or even disappears because the macromolecules of higher molar mass are more strongly contracted than those of lower molar mass. After a certain conversion of pendant vinyl groups, the influence of the intermolecular reaction on $[\eta]$

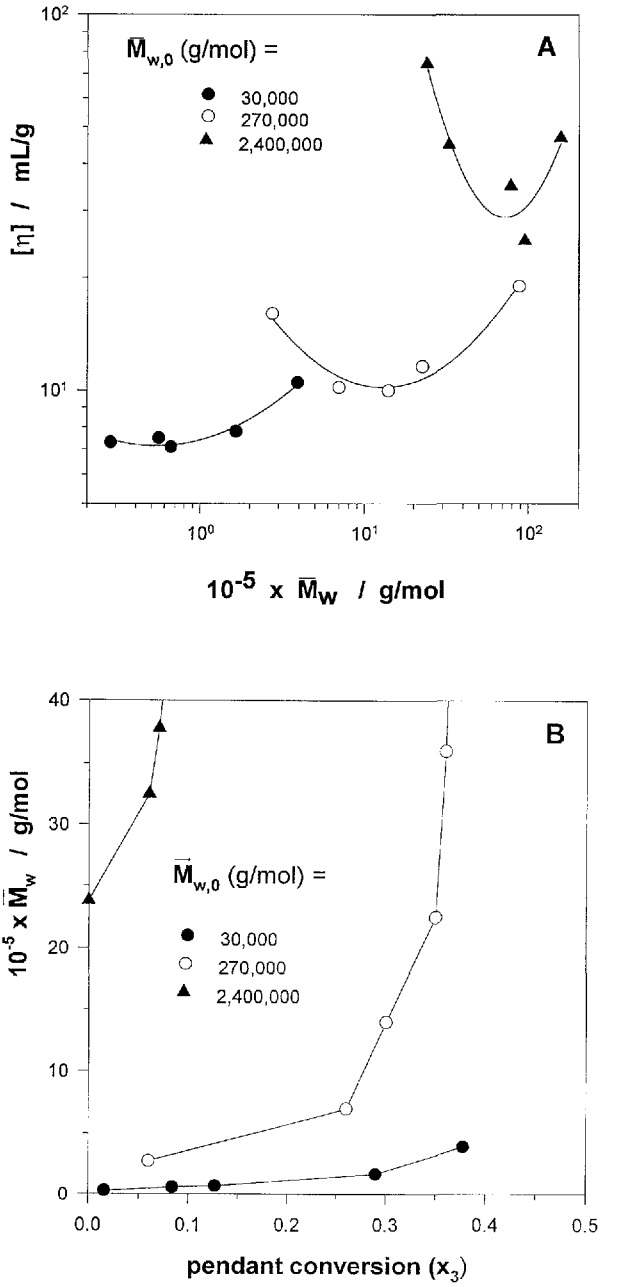


Fig. 39. Relation between [η] and $\bar{M}_w$ in the course of crosslinking of PVS. B: Increase of $\bar{M}_w$ with the conversion of pendant vinyl groups during crosslinking of PVS. PVS concentration = 0.35 mass %. Temperature = 70 °C. Molar masses of the starting PVS, $\bar{M}_{w,0}$ are shown in the figures. [Reproduced from Ref. 218 with permission, Hüthig & Wepf Publ., Zug, Switzerland].

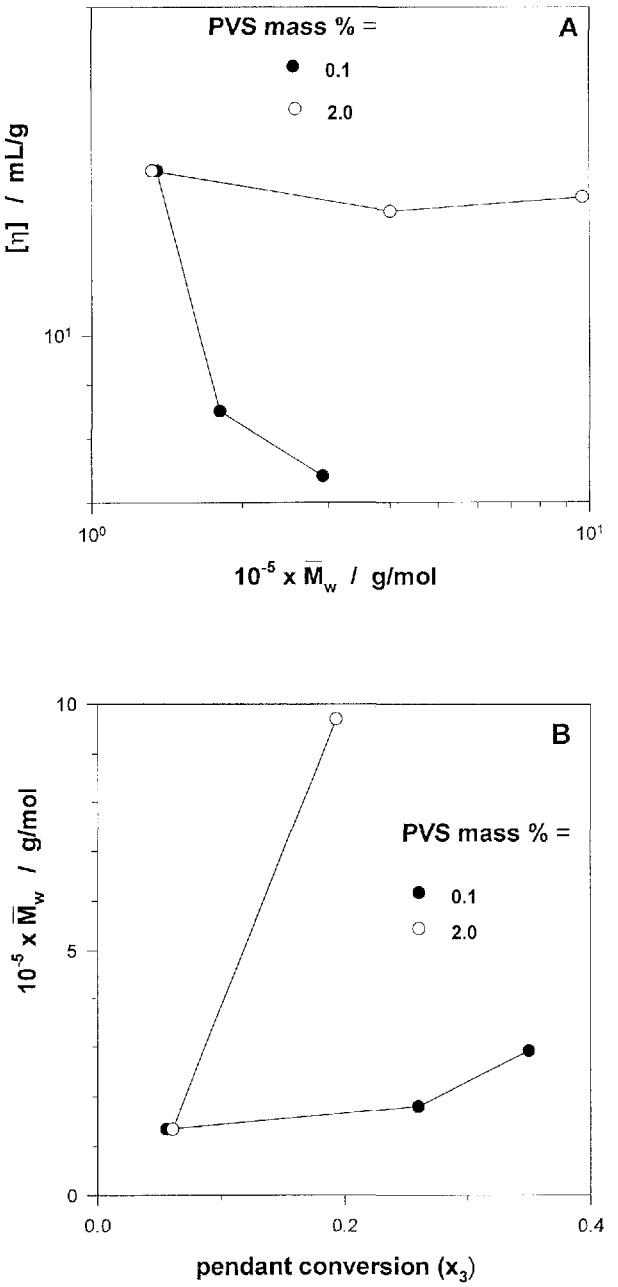


Fig. 40. **A:** Relation between [η] and $\overline{M}_w$ during crosslinking of PVS. **B:** Increase of $\overline{M}_w$ with the conversion of pendant vinyl groups during crosslinking of PVS. Molar mass of starting PVS, $\overline{M}_{w,0}$ = 135000 g/mol. Temperature = 70 °C. The PVS concentrations are shown in the figures [217].

dominates and aggregates are formed. The transition from microgels to a macrogel is indicated by an abrupt increase of $\overline{M}_w$ with the increase of the conversion of pendant vinyl groups x_3 (Fig. 39B).

The degree of initial dilution strongly influences the extent of cyclization during the formation of microgels [217]. As seen in Fig. 40A, the slope at the beginning of the $[\eta]/M_w$ curves becomes steeper when the concentration of PVS is decreased from 2.0 to 0.1 mass % which means that cyclization is much more favored. As a result, the onset of the fast increase of the molar mass and the gel point are shifted to higher conversions of pendant vinyl groups (Fig. 40B). It was also shown that the extent of cyclization increases and the point of the macrogel formation is shifted towards higher conversions of pendant vinyl groups when the chain transfer constant of the solvent used in polymerization increases [217]. This result confirms the observations of Chen et al. in 1,4-DVB/MMA copolymerization [214].

The solvating power of the solvent used in polymerization also strongly influences the rate of cyclization. Batzilla crosslinked PVS in a series of toluene/methanol mixtures of increasing content of the non-solvent methanol and measured the initial conversion rate of pendant vinyl groups, which corresponds to the rate of cyclization [217]. As seen in Fig. 41, this rate increases very rapidly

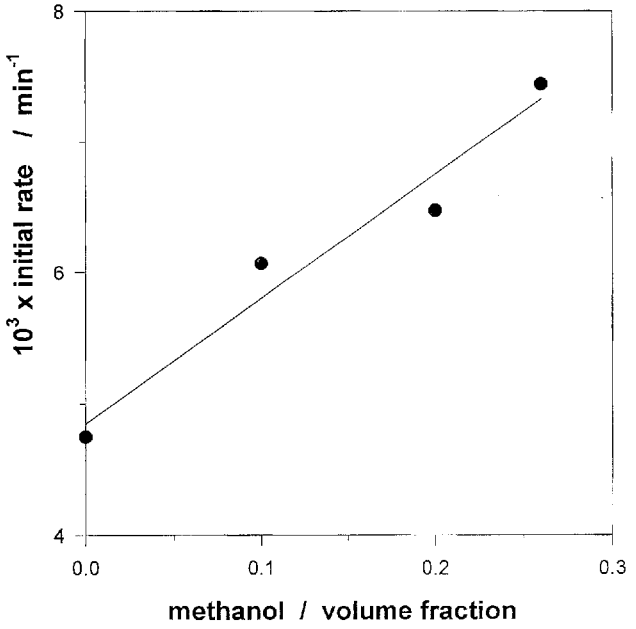


Fig. 41. Initial rate of the conversion of pendant vinyl groups during crosslinking of PVS shown as a function of the volume fraction of methanol in the toluene/methanol mixture [217]. PVS concentration = 0.30–0.35 mass %, initial molar mass of PVS = 170000 g/mol, temperature = 70 °C.

with the volume fraction of the non-solvent. In poor solvent mixtures the polymer coils are contracted which necessarily increases the local concentration of pendant vinyl groups within the polymer coils. Therefore, the probability of cyclization increases. Under identical conditions, however, macrogelation occurs earlier in poor than in good solvents [217]. The delayed gelation in good solvents was also observed by Matsumoto in several polymerization systems [30]. He explained this observation by the influence of a thermodynamically excluded volume effect on intermolecular crosslinking. Accordingly, the reactivity of pendant vinyl groups in large molecules is probably much lower in good than in poor solvents due to the excluded volume of the molecule. This excluded volume effect seems to dominate when macrogelation occurs at low conversions, i.e. when the concentration of PDS in the transition region to the macrogel is rather low. Similar results were reported with 1,4-DVB/MMA microgels dissolved in benzene-methanol mixtures [214, 215, 219, 220]. By varying the solvent composition of these solvent mixtures, Ishizu et al. measured the rate of cyclization and intermolecular crosslinking in the copolymerization of 1,4-DVB and MMA [219]. The rate of cyclization increased with the content of methanol in the solvent mixture. However, with a methanol fraction of 50%, the rate of cyclization became extremely small. On the other hand, the dependence of the rate of intermolecular crosslinking on the solvent quality was maximal at a methanol fraction of 0.1.

With regard to these results, experiments were designed to prepare intramolecularly crosslinked macromolecules by starting from linear polymers with a negligible number of intermolecular links [217]. In Table 2 the reaction conditions as well as the properties of PVS before and after the reaction are collected. After a reaction time of 25 min, $[\eta]$ decreased to half of the initial value whereas only a slight change of $\overline{M}_w$ could be detected by light scattering. It was calculated that the ratio of cycles to intermolecular links in the product was 500:1. Therefore, the reaction product can be considered as a primary particle, i.e. an intramolecularly crosslinked macromolecule. It is obvious that such intramolecularly crosslinked macromolecules may be formed during RCC of vinyl/divinyl monomer mixtures at zero monomer conversion. The intermolecular crosslinking between these molecules and the subsequent multiple crosslinking lead to the formation of microgels.

Table 2. Intramolecular crosslinking of PVS [217]. Reaction conditions: PVS concentration = 0.975 mass %; AIBN concentration = 1.65×10^{-3} M; temperature = 70 °C; n-butylmercaptan (chain transfer agent) concentration = 20 mL/L; reaction time = 25 min. The $\overline{M}_w$ and $\overline{M}_n$ were measured by light scattering and membrane osmometry respectively.

POLYMER :		PVS	PRODUCT
x_3		0	0.27
$\overline{M}_w$	[g.mol^{-1}]	120,000	160,000
$\overline{M}_n$	[g.mol^{-1}]	32,000	32,000
$[\eta]$	[mL/g^{-1}]	16	8

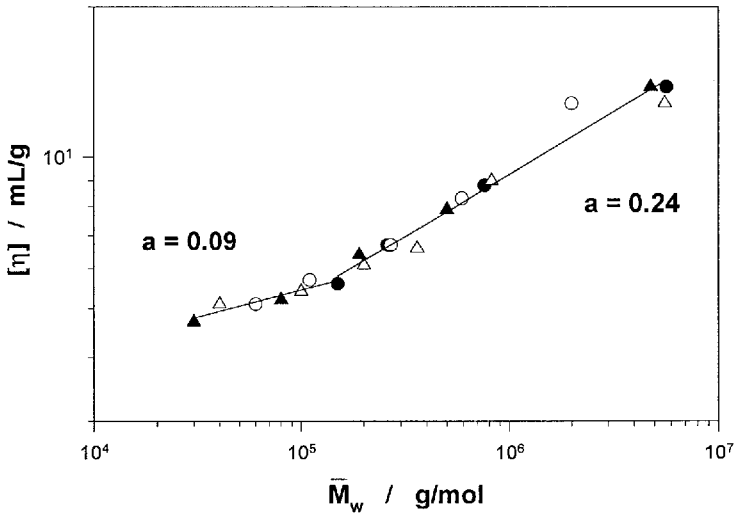


Fig. 42. Relation between $[\eta]$ and $\overline{M}_w$ of 1,4-DVB microgels synthesized at initial monomer concentration 5 ( ), 2 (○), 1 (▲), and 0.5 g/100 mL (△). AIBN concentration = 8×10^{-3} M, temperature = 70 °C. [Reprinted with permission from Ref. 209, Copyright 1995, American Chemical Society].

6.4 Characteristics of Microgels

The compact structure of microgels which is due to extensive cyclization and multiple crosslinking, manifested itself in the $[\eta]/\overline{M}_w$ plots. Figure 42 shows the relation between $[\eta]$ and $\overline{M}_w$ for the microgels obtained by RCC of 1,4-DVB with different monomer concentrations [209, 212]. The exponent a of the Mark-Houwink equation, calculated for each monomer concentration, decreases gradually from 0.25 to 0.20 as the dilution increases. Moreover, the average value of the exponent a is close to zero for $\overline{M}_w < 10^5$ due to the predominant cyclization and multiple crosslinking, and becomes 0.24 above this molar mass, compared with the value a = 0.7 for linear polystyrene in benzene. Thus, the exponent a agrees well with previous results about the extent of cyclization. Figure 43 shows the same plot with a slope of 0.21 ± 0.01 for different initiator concentrations and 1,4-DVB/S ratios. This value of the exponent is close to the value of 0.25 reported by Zimm et al. for 1,4-DVB/S copolymers [162]. For the same system, Antonietti and Rosenauer found an exponent 0.38, which deviates distinctly from the first two values [221]. Their microgels were prepared by polymerization of t-DVB/S mixtures in dilute benzene solutions over a time of 20 days at 70 °C with repeated additions of AIBN. Obviously, these authors obtained a rather heterogeneous mixture of branched polystyrene and microgels which explains the high value for the exponent a.

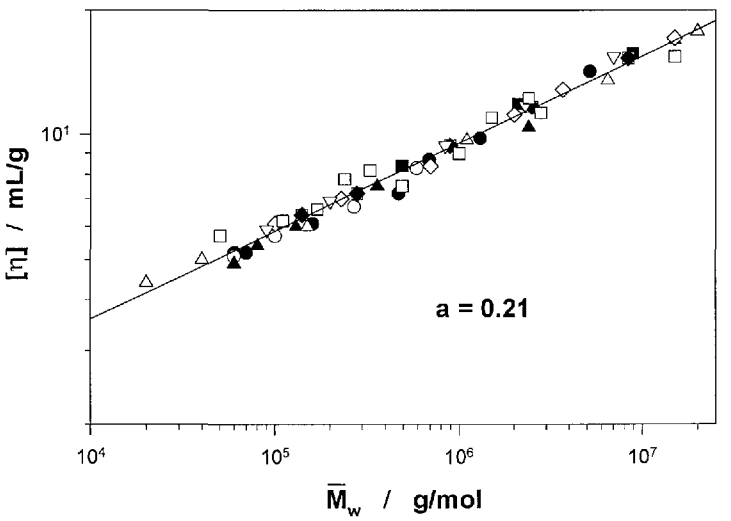


Fig. 43. [η] versus $\overline{M}_w$ of 1,4-DVB microgels obtained under various reaction conditions: 1) Pure 1,4-DVB microgels: Initial monomer concentration = 2 g/100 mL, temperature = 70 °C, AIBN concentration = 2.6 ( ), 8(○), 16 (▲), and 32mM (△). 2) 1,4-DVB/S microgels: Initial monomer concentration = 5 g/100 mL, temperature = 70 °C, AIBN concentration = 2.6 mM, 1,4-DVB mass % = 100 (■), 80 (□), 70 (◆), 60 (◇), 40 (▽), and 20 (▼). [Reprinted with permission from Ref. 209, Copyright 1995, American Chemical Society].

In Figure 44, $[\eta]/\overline{M}_w$ plots for various microgels formed in an emulsion and in solution are schematically illustrated. Emulsion polymerization yields polymer gel spheres with a constant density, if the amount of crosslinker in the monomer mixture is higher than 10 %. If the crosslinking density of the microgels increases or if the quality of the swelling solvent decreases, [η] decreases but never attains the value of rigid spheres. Thus these microgels swell to some extent. However, because microgels formed in solution, compared to coils of linear macromolecules, are also contracted, the dependence of [η] on their molar mass indicates a density fluctuation within particles. Probably dangling chains on the microgels or loosely crosslinked regions between the primary particles within a microgel aggregate may cause the observed deviations. Only in the region of $\overline{M}_w < 10^5$, where the intermolecular reactions are insignificant, is the exponent a close to zero for microgels formed in solution and they behave like those formed in emulsion.

In t-DVB/S copolymerization, Antonietti and Rosenauer isolated microgels slightly below the macrogelation point [221]. Using small angle neutron scattering measurements they demonstrated that these microgels exhibit fractal behavior, i.e. they are self-similar like the critically branched structures formed close to the sol-gel transition.

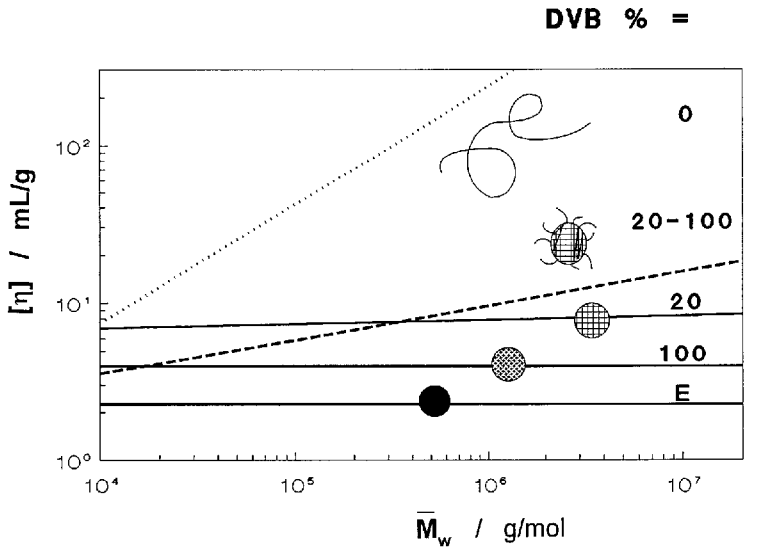


Fig. 44. Schematic representation of $[\eta]/\overline{M}_w$ plots for microgels formed in emulsion (solid lines) and in solution (dashed line). Solvent = toluene. Temperature = 25°C. The dotted line represents the plot of linear polystyrene. The 1,4-DVB contents are given in the figure. E = Einstein equation.

7
Microgel Formation by Anionic Polymerization

Anionic polymerization is a powerful method for the synthesis of polymers with a well defined structure [222]. By careful exclusion of oxygen, water and other impurities, Szwarc and coworkers were able to demonstrate the "living" nature of anionic polymerization [223, 224]. This discovery has found a wide range of applications in the synthesis of model macromolecules over the last 40 years [225–227]. Anionic polymerization is known to be limited to monomers with electron-withdrawing substituents, such as nitrile, carboxyl, phenyl, vinyl etc. These substituents facilitate the attack of anionic species by decreasing the electron density at the double bond and stabilizing the propagating anionic chains by resonance.

For the synthesis of reactive microgels, anionic polymerization has received less attention compared to the other methods. This is due to the experimental difficulties involved in this synthesis. For instance, the isolation of the polymers in the microgel stage is difficult because anionic polymerization proceeds at very high rates. However, anionic polymerization is advantageous for preparing predetermined and well-defined network structures. Moreover, the simple kinetics allows a better insight into the complex mechanism of microgel formation.

Among the divinyl monomers, 1,4-DVB and EDMA are the most extensively studied monomers for microgel formation by anionic polymerization. Com-

pared to EDMA, 1,4-DVB is less reactive because of its relatively weak electron-withdrawing substituent. Thus, strong nucleophiles such as alkyl carbanions are required to polymerize 1,4-DVB. EDMA can easily be polymerized anionically by using weaker nucleophiles such as alkoxide ions, although various side reactions are possible between anions and the ester groups of the monomer or of the growing polymer [228]. A variety of initiators have been used to initiate the anionic polymerization of divinyl monomers. Depending on the solvent used, the reactions may proceed in homogeneous or heterogeneous solutions. Various factors are known to influence the structure of the resulting polymers and their properties. The following discussion summarizes the experimental results of synthesizing reactive microgels by anionic methods and the conditions of their formation. The initiator concentrations will be expressed as mol % of the initial content of monomers. The content of pendant unsaturated groups of the polymers is expressed as the fraction of the tetrafunctional monomer units in the polymer that bear a pendant unsaturated group.

7.1
1,4-Divinylbenzene (1,4-DVB)

Both vinyl groups of 1,4-DVB have equal reactivities but after one of them has reacted, the remaining vinyl group (pendant group) has a lower reactivity. Worsfold showed that in anionic polymerization the reactivity of pendant vinyl groups is ten times smaller than the reactivity of vinyl groups of the 1,4-DVB monomer [229]. This suggests that at the beginning of the polymerization in dilute solutions almost linear poly(4-vinylstyrene) (PVS) chains must be formed, which then branch and, as polymerization proceeds, are finally connected to an infinite network.

On copolymerization of DVB containing 45% ethylstyrene and on terpolymerization of this mixture with 75% styrene, using a Ziegler-Natta catalyst and aliphatic or aromatic solvents, D'Alelio and Brüschweiler [384] obtained soluble polymers with an average intrinsic viscosity of 0.1–0.11. By using the K and a values of polystyrene they calculated a molar mass of 5000–6000, corresponding to an average degree of polymerization of 40–50. As the titration with bromine indicated that only one double bond of each polymerized DVB unit reacted, it was concluded that the polymers had a linear structure. However, considering the low $[\eta]$ values, it cannot be excluded that these co- and terpolymers were very weakly crosslinked microgels.

Dusek [385] found that on crosslinking of these soluble polyunsaturated polymers, the crosslink densities were much lower than those of corresponding polymers obtained by direct polymerization of the monomer mixtures. This result indicates a strong sterical hindrance of pendant vinyl groups.

Hiller and Funke obtained easily dissolvable linear macromolecules of PVS by anionic polymerization of 1,4-DVB up to conversions of 80–90% [230, 231]. In these experiments very low concentrations of *n*-butyl lithium (*n*-BuLi) were used and tetrahydrofuran (THF) as solvent. The reactions were carried out at –78 °C and for 7 min. The contents of pendant vinyl groups in the polymer were determined by infrared spectroscopy, mercury-II-acetate addition and catalytic

hydrogenation with tris(triphenylphosphin)-rhodium-I-chloride as catalyst. These investigations indicated that each 1,4-DVB unit in the polymer had approximately one pendant vinyl group. The $\overline{M}_w$ of PVS thus prepared, varied, between 6×10^4 and 40×10^4 g/mol, depending on the initiator concentration [230–232]. The [η] of the polymers in toluene at 20 °C varied between 23.5 and 165 ml/g. As seen in the previous section, PVS macromolecules thus obtained are excellent multifunctional macromonomers for studying cyclization and multiple crosslinking in radical polymerization. According to Tsuruta et al., almost linear PVS can be also prepared in THF if the polymerization is initiated with lithium diisopropylamide in the presence of an excess diisopropylamine [233–235]. The molar mass of these polymers, however, is relatively low ($\overline{M}_w < 10^5$ g/mol) due to the chain transfer reactions of the free amine in the reaction medium.

Hiller and Funke extensively investigated the change of the polymer structure as a function of the monomer and the initiator concentration in various solvents [231]. The content of pendant vinyl groups in the polymer was about 100% for *n*-BuLi concentrations below 2 mol % and for the whole range of the monomer concentration studied (20–100 g/l). The content of pendant groups decreased when the *n*-BuLi concentration increased and approached 80 % in the transition region of a soluble polymer to a macrogel. As seen in Fig. 45, the decrease of pen-

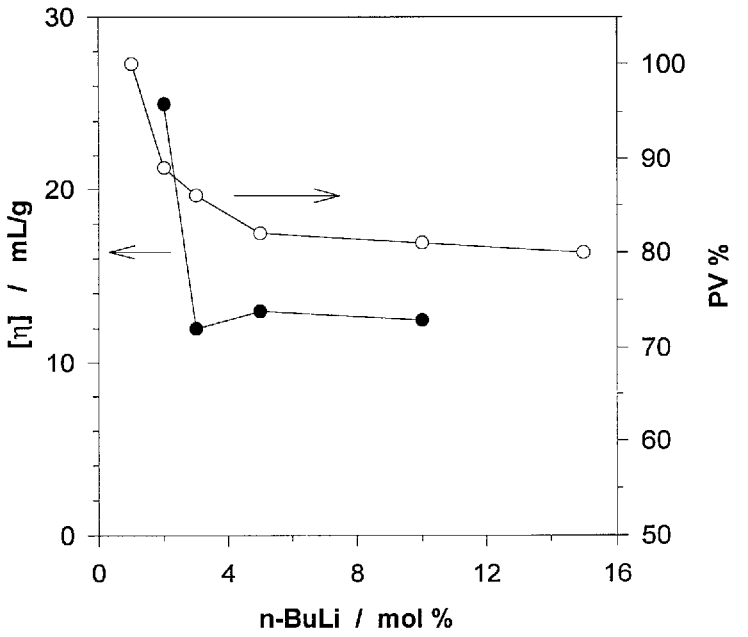


Fig. 45. [η] and content of pendant vinyl groups of polymers shown as a function of the initial n-BuLi concentration in the anionic polymerization of 1,4-DVB in THF. Initial 1,4-DVB concentration = 20 g/L. Reaction temperature = –78 °C. Reaction time = 7 min. [Reproduced from Ref. 231 with permission, Hüthig & Wepf Publ., Zug, Switzerland].

dant vinyl groups and of [η] is rapid up to 3 mol % *n*-BuLi, indicating an increasing tendency of the polymer chains to cyclization. Later on the content of pendant groups decreases further, but [η] increases only slightly because of an increasing extent of intermolecular crosslinking. For *n*-BuLi contents above 15 mol %, insoluble aggregates were obtained. Thus, a gradual change from a linear to a crosslinked structure of the polymer can be achieved by with increasing the concentration of *n*-BuLi. Intramolecular crosslinking, leading to the formation of microgels, play an important role at medium *n*-BuLi concentrations . The [η] of the microgels were in the range of 12–14 ml/g and were almost independent of the molar mass (Fig. 46).

The pendant vinyl groups of the microgels react with mercury-II-acetate at different rates, depending on their location (Fig. 47). At the beginning vinyl groups located at the surface of microgels react fast. Then a low, diffusion-controlled reaction of the vinyl groups within the microgels takes place.

Electron micrographs of microgels at a magnification of ×152|000 show that their shape is spherical or sometimes irregular with diameters of 3–30 nm [230]. Because microgels are reactive crosslinked macromolecules, the particle growth during the polymerization may also proceed by aggregation of these primarily formed, reactive microgels, and larger, irregularly shaped aggregates of microgels are produced. Electron micrographs of macrogels showed that they are com-

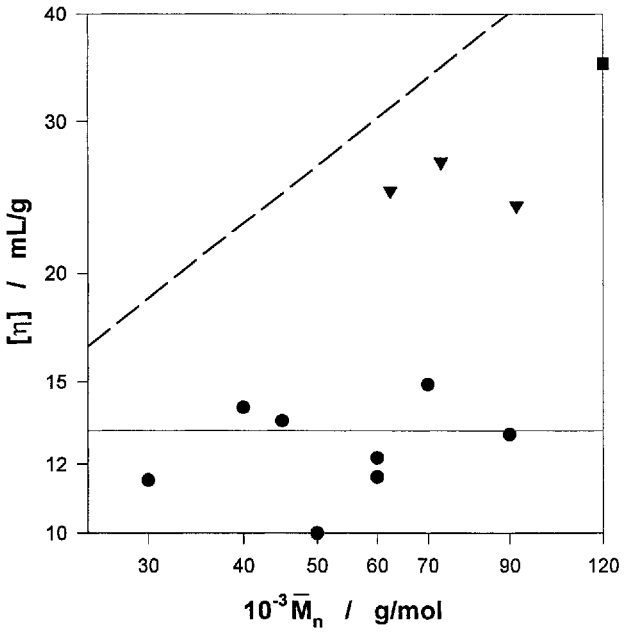


Fig. 46. Dependence of [η] on the $\overline{M}_n$ of polymers prepared by anionic polymerization of 1,4-DVB in THF. The symbols represent linear (■); branched (▼) and microgel ( ) structures. The dashed line represents the [η]/$\overline{M}_n$ relationship of anionically prepared polystyrene. [Reproduced from Ref. 231 with permission, Hüthig & Wepf Publ., Zug, Switzerland].

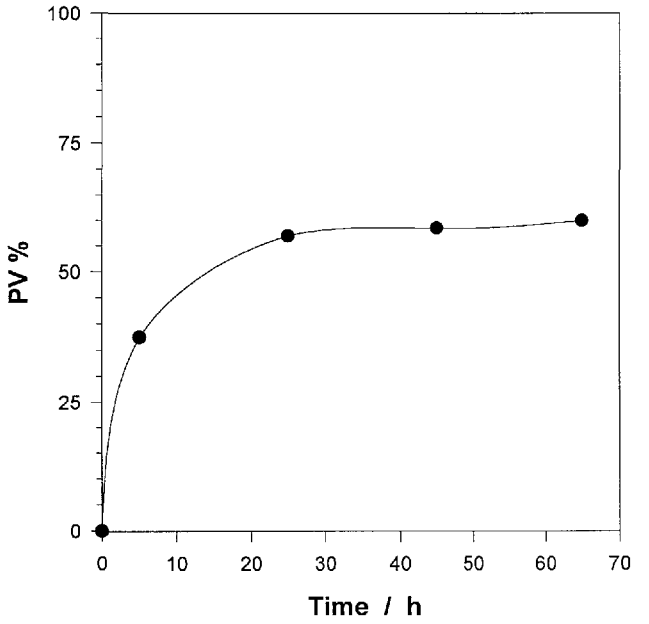


Fig. 47. Conversion of pendant vinyl groups (PV) in 1,4-DVB microgels shown as a function of the reaction time with mercury-II-acetate [230]. Initial monomer and initiator concentrations used for the synthesis of the microgels are 30 g/L and 4 mol % respectively.

posed of small particles with the same size and shape as those of microgels. This directly confirms that microgels are intermediates in the formation of highly crosslinked, macroscopic networks.

In Fig. 48, the regions of the formation of linear or branched polymers, microgels and macrogels are shown as a function of the concentration of 1,4-DVB and of *n*-BuLi. Reactive microgels can be obtained at a monomer concentration below 50 g/l and between 3 and 16 mol % of *n*-BuLi. The polymer structure approaches that of a macrogel when the concentration of 1,4-DVB or *n*-BuLi is increased.

Anionic polymerization of 1,4-DVB by *n*-BuLi leading to the microgels was also reported by Eschwey et al. [236, 237]. In their experiments, *n*-BuLi was used at very high concentrations of 17 and 200 mol % of the monomer. Contrary to the results of Hiller and Funke [231], they observed a transition from microgel to macrogel with decreasing *n*-BuLi concentration. Similar results were also reported by Lutz and Rempp [238]. They used potassium naphthalene as the initiator of the 1,4-DVB polymerization and THF as the solvent. Soluble polymers could only be obtained above 33 mol % initiator, whereas below this value macrogels were obtained as by-products.

The opposite effects of the initiator on the structure of 1,4-DVB polymers in a range of low (1–16 mol %) and a high (17–200 mol %) concentration of 1,4-DVB were explained by a kinetic model of anionic polymerization of 1,4-DVB [239]. Calculations indicated that, at low concentrations of the initiator, the poly-

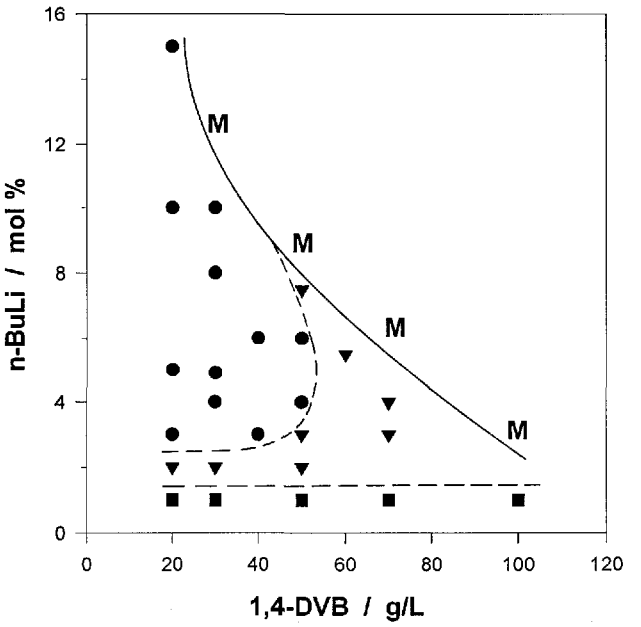


Fig. 48. Dependence of the polymer structure on the initial concentrations of n-BuLi and 1,4-DVB in the anionic 1,4-DVB polymerization in THF. The symbols represent linear (■); branched (▼); microgel ( ) and macrogel (M) structures. [Reproduced from Ref. 231 with permission, Hüthig & Wepf Publ., Zug, Switzerland].

merization is slow, and an increase of the concentration of *n*-BuLi leads to the formation of a macrogel because polymerization and crosslinking are accelerated. At high concentrations of the initiator, the polymerization and crosslinking are very fast, but the length of the primary chains limits the crosslinking density. Therefore, within this range macrogels are formed earlier with decreasing *n*-BuLi concentration because the length of the primary chains increases. The calculated dependence of the polymer structure on the initial concentration of monomer and initiator is shown in Fig. 49. The solid curve which represents the transition from microgels to a macrogel, resembles the experimental curve for the range of 1 to 16 mol % *n*-BuLi (Fig. 48).

The solvent used in the anionic polymerization of 1,4-DVB by *n*-BuLi also has an important effect on the polymer structure. If polymerization reactions are carried out in benzene/THF mixtures, the onset of macrogelation can be retarded by increasing the THF fraction in the solvent mixture [230]. Hexane, that is a solvent for the monomer but a precipitant for the resulting polymer, was not suitable because an insoluble aggregate was formed within a few minutes [230]. For hexane /THF mixtures with equal volumes, the conditions for the synthesis of a soluble polymer depends on the concentrations of 1,4-DVB and *n*-BuLi (Fig. 50). The course of the curve in the transition region from a soluble polymer to a macrogel is similar to that shown in Fig. 48 for *n*-BuLi/THF.

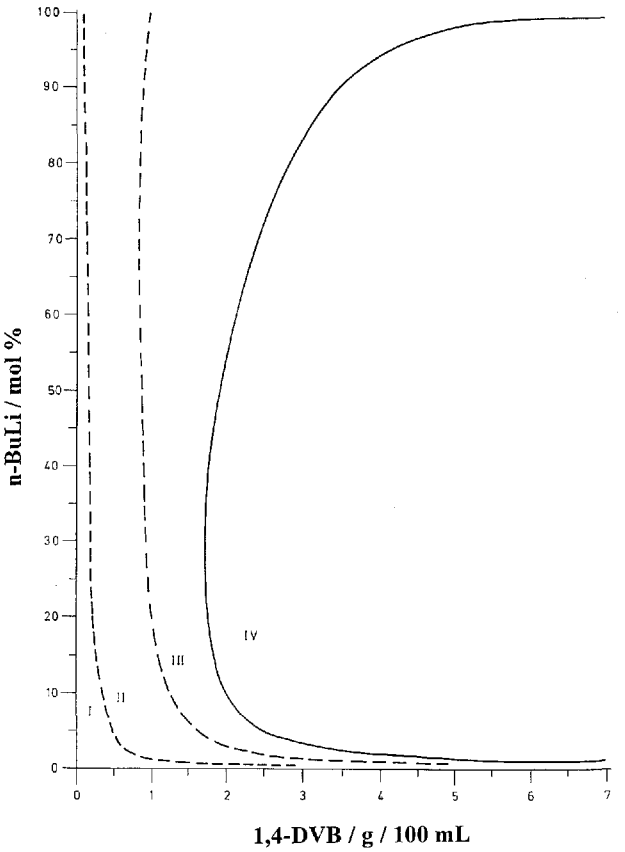


Fig. 49. Calculated dependence of the polymer structure on the initial 1,4-DVB and n-BuLi concentrations in the anionic 1,4-DVB polymerization. The numbers I to IV represent the region for the formation of linear, branched, microgel and macrogel structures, respectively. The solid and dashed curves represent the transition regions between these structures. [Reproduced from Ref. 239 with permission, Hüthig & Wepf Publ., Zug, Switzerland].

The use of living polymers for initiating the anionic polymerization of 1,4-DVB may also lead to the formation of reactive microgels, which have a shell of linear polymers. This method is known to be applied in the synthesis of star-shaped polymers, where only a small amount of 1,4-DVB is used as crosslinking agent for the living chains [240–243]. In this polymerization of 1,4-DVB the nucleus of a star-shaped polymer is formed with linear polymer chains bound to its as branches. The mass of the nucleus of star-shaped polymers is usually negligible (1–5 mass %). Therefore these polymers may be considered as a limiting case of reactive microgels which are enveloped by linear polymer chains. Taromi and Rempp reported that the size of the nuclei in star-shaped polymers can be enlarged when more 1,4-DVB is used [244]. They applied the same

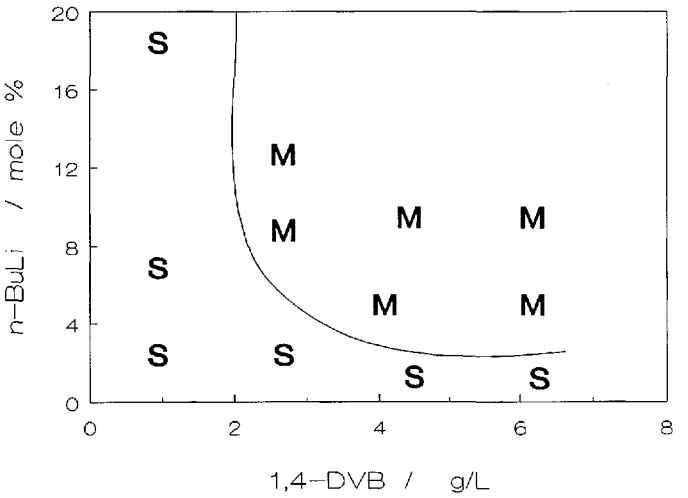


Fig. 50. *Dependence of the polymer structure on the initial concentrations of n-BuLi and* 1,4-DVB in the anionic polymerization of 1,4-DVB in a hexane / THF (1/1) mixture [230]. S = soluble polymer; M = macrogel. The solid curve represents the soluble polymer – macrogel transition region.

method as that for the synthesis of real star polymers: addition of 1,4-DVB to a solution of polystyryl lithium in benzene or in THF. The reaction proceeded in a homogeneous solution and reactive microgels ("porcupine polymers") were obtained which consisted of about 30–34 mass % of nuclei. The residual mass consisted of polystyrene chains attached to the surfaces of the nuclei (10–90 chains per nucleus). No gelation of the reaction mixture was observed even at very high concentrations of 1,4-DVB. This indicates that the polystyrene chains prevent the reaction between the pendant vinyl groups at the surface of the nuclei.

Another approach to the synthesis of reactive microgels with living polymer chains is anionic dispersion polymerization. This method, which was thoroughly reviewed by Barret [245], is a modified precipitation polymerization [246]. For applying this method to the synthesis of microgels the divinyl monomer and the living polymer must be soluble in the dispersion medium, while its block copolymer must be insoluble. Okay and Funke initiated the polymerization of 1,4-DVB by using living poly(4-*tert*-butylstyrene) (PBS) at 50 °C in *n*-heptane [247, 248]. Heptane is known to be a good solvent for PBS, but a non-solvent for polystyrene or PVS. The polymerization was thus initially homogeneous, but the growing block copolymer chains, consisting of PBS and PVS blocks, precipitated from the solution after attaining a critical size (Fig. 51). Further polymerization and crosslinking proceeded in the separated phase of the block copolymer, and reactive 1,4-DVB microgels were obtained which were enveloped by PBS chains. The chains of the living polymer act as the initiator for the polymerization of 1,4-

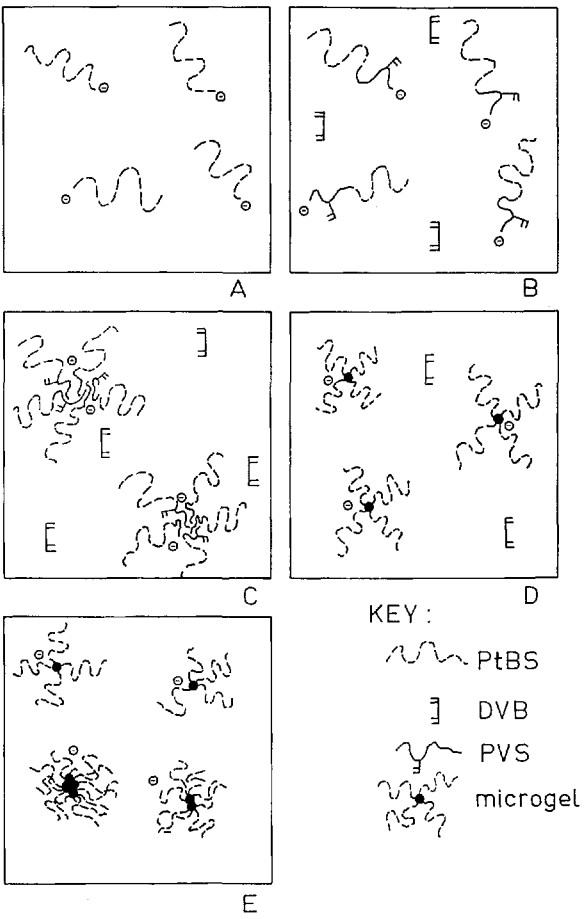


Fig. 51. Schematic illustration of the mechanism of microgel formation in the anionic dispersion polymerization of 1,4-DVB initiated by living PBS chains in heptane. [Reprinted with permission from Ref. 247, Copyright 1995, American Chemical Society].

DVB and also as steric stabilizers for the separated particle phase. By this method reactive microgels with nuclei fractions up to 30–35 mol % (25–30 mass %) could be prepared without macrogel formation [247]. The microgels had $\overline{M}_w$ up to 30×10^6 g/mol and possessed 10–5000 PBS chains per nucleus, which were packed closely together at the surface. In Fig. 52, the [η] of these microgels are plotted against their mol fractions of 1,4-DVB, (n_{DVB}). Though the $\overline{M}_w$ of the microgels is 20–6000 times higher than that of linear, soluble macromolecules ($n_{DVB} = 0$), their [η] does not differ much. The [η] of these microgels was only 1.5–3 times higher than those of linear macromolecules . The higher the [η] of the soluble macromolecules, the higher was also the [η] of the resulting microgels. Thus, the length of PBS chains attached to the surface of the nuclei controls

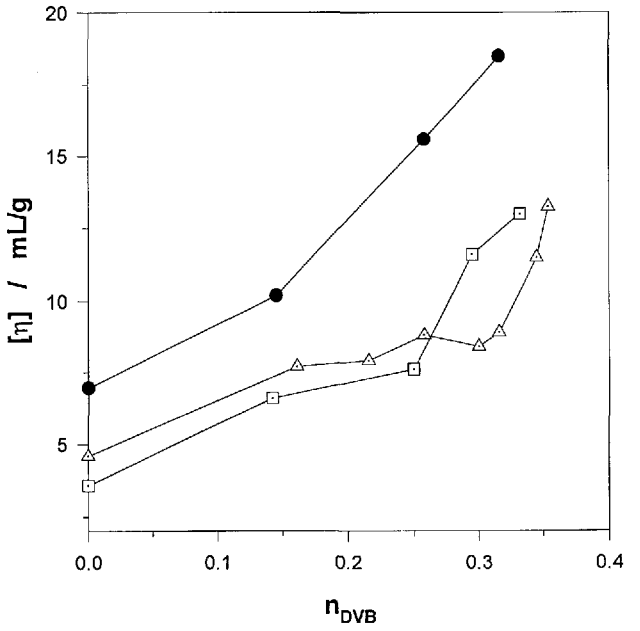


Fig. 52. [η] of the microgels shown as a function of mole fraction of nuclei in the microgels n_{DVB} in the anionic dispersion polymerization of 1,4-DVB. $\overline{M}_n$ of living PBS = 2700 – 3400 (□); 4000 – 5000 (△); 13000–16500 ( ). [Reprinted with permission from Ref. 247, Copyright 1995, American Chemical Society].

the [η] of the microgels. The hydrodynamic volume of these microgels could be regulated by the length of living PBS used for their synthesis.

Pille and Solomon investigated the formation of the above mentioned microgels using gel-permeation chromatography with an on-line light scattering detector [249]. They showed that the primary particles appear very early in the reaction and microgels are formed by interparticular reactions of the primary particles.

7.2
1,3-Divinylbenzene (1,3-DVB)

Kast and Funke studied the anionic polymerization of 1,3-DVB and compared the results with those obtained using 1,4-DVB [250]. The polymerization was carried out under constant conditions (solvent = THF; initiator = *n*-BuLi; temperature = –78 °C). Significant differences between the behavior of both isomers were observed.

1) Polymerization of 1,3-DVB is much faster than 1,4-DVB. Conversion of 1,3-DVB was complete within seconds, whereas in the case of 1,4-DVB the conversion of the monomer was 80–90 % after 7 min.

2) The content of pendant vinyl groups of 1,3-DVB microgels was found to be 50–70% and almost independent of the monomer conversion. Linear or slightly branched 1,3-DVB polymers could not be isolated. In the case of 1,4-DVB, the content of pendant vinyl groups was about 100% at low conversions and decreased to 80% with increasing conversion. This comparison clearly shows that in the polymerization of 1,3-DVB cyclization dominates and that the reactivity of the pendant vinyl groups of 1,3-DVB units is much higher than of 1,4-DVB units.
3) Under the same reaction conditions macrogelation occurs later in the polymerization of 1,3-DVB. Moreover, the $[\eta]$ of the microgels from 1,3-DVB is much smaller than that from 1,4-DVB. The exponent a of Mark-Houwink equation for the 1,3-DVB polymers in toluene was found to be only 0.25 [250] and 0.29 [251] compared with 0.48 for 1,4-DVB polymers obtained under similar reaction conditions [230]. The delay of the gel point and the small hydrodynamic volumes of 1,3-DVB microgels, compared with 1,4-DVB microgels also illustrate that the extent of cyclization is much higher in 1,3-DVB polymerization.

7.3 Ethylene Glycol Dimethacrylate (EDMA)

The methacrylate groups in EDMA do not interact electronically. Therefore, the reactivity of pendant methacrylate groups at the polymer backbone should be the same as that of the monomers [100]. Moreover, the crosslinks formed with EDMA are less bulky and more flexible than those with 1,4-DVB. Therefore, the pendant methacrylate groups should react more efficiently in polymerization and both polymerization and crosslinking may occur simultaneously at the beginning of the reaction. However, an equal reactivity of methacrylate groups in polymerization of EDMA is only valid in systems without steric hindrance. With increasing density of intra- and/or intermolecular crosslinking and with decreasing mobility of the polymer chains, the reactivity of pendant methacrylate groups may gradually decrease during the formation of microgels or within microgels with increasing distance of pendant groups from surface to the center of microgels.

Beer used sodium methylate/methanol as initiator system for the anionic polymerization of EDMA [252]. Since this initiator system yields oligomers in the polymerization of methyl methacrylate [253], it was aimed to synthesize EDMA oligomers and finally obtain EDMA microgels by their aggregation. However, insoluble macrogels were obtained due to the high reaction rate. With potassium *tert*-butylate and dibenzo-18-crown ether-6, insoluble products were also formed within a few seconds [252]. Initiation of the EDMA polymerization by *n*-BuLi in THF resulted in various side-reactions between *n*-BuLi and the ester groups of EDMA [254–256] as was observed in the anionic polymerization of methyl methacrylate [257–259]. When *n*-hexane was used as solvent, insoluble macrogels were obtained immediately after the addition of the monomer to the initiator solution. The best results were obtained in toluene, a good non-polar solvent for EDMA and also for the resulting polymer [256]. The polymerization

in toluene was carried out at 20 °C and with an initial monomer concentration of 78 g/l. The fraction of pendant methacrylate groups was between 51 and 59% and almost independent of conversion and molar mass. This interesting feature of anionic EDMA polymerization was also observed by Galina et al. in radical polymerization of EDMA [173].

These results suggest that reactive microgels are already formed at the very beginning of polymerization as a consequence of dominating cyclization. Thus, contrary to the anionic polymerization of 1,4-DVB, linear polymers bearing methacrylate groups do not appear in the EDMA polymerization. The higher extent of cyclization in EDMA microgels is also reflected by their "molecular" swelling ratios. Antonietti et al. showed that the swelling ratio of EDMA/S microgels formed in a microemulsion was much higher than expected from the chemical crosslink density [95].

Straehle observed two distinct reaction stages in the anionic polymerization of EDMA (Figs. 53 and 54) [254]. In the first stage, the rate of polymerization was rapid and after 3 min the polymer yield increased up to 25.8%. During the same period $\overline{M}_w$ increased only slightly. This indicates a low rate of intermolecular crosslinking. Moreover, the relatively low content of pendant methacrylate groups (59%) indicates strong cyclization. The exponent of the $\langle s^2 \rangle_z / \overline{M}_w$ relation was between 0.66 and 0.71 which indicates a spherical shape of the polymers. It must be concluded, therefore, that during the first stage of the reaction, spherical, intramolecularly crosslinked macromolecules, i.e. reactive microgels, are formed. In the second stage of the reaction (after 3 min) the yield of the polymer increases slowly and after 7 min reaches 36.5%, but $\overline{M}_w$ increases rapidly as the reaction proceeds and after 8 min the first macrogel particles appear [254]. Thus, during this stage of the reaction the rate of monomer consumption decreases and mainly intermolecular crosslinking occurs. The transition from the first to

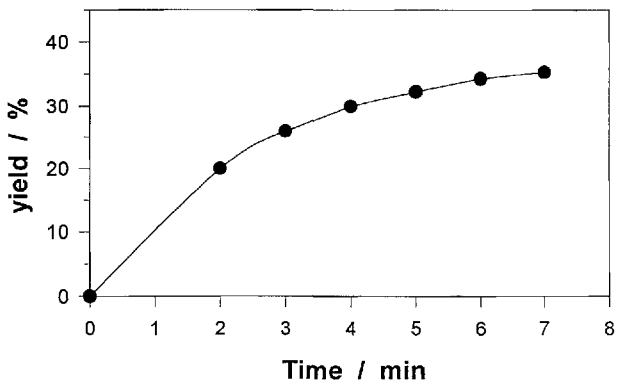


Fig. 53. Dependence of the polymer yield on the reaction time in the anionic polymerization of EDMA in toluene by n-BuLi [254]. Initial monomer and initiator concentrations are 78 g/L and 5.78 mol %, respectively. Reaction temperature = 20 °C.

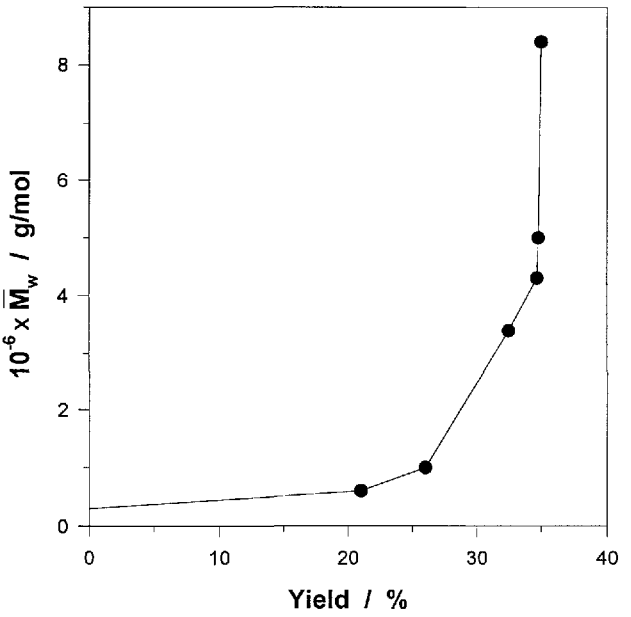


Fig. 54. Dependence of $\overline{M}_w$ of the microgels on the polymer yield in the anionic polymerization of EDMA in toluene by n-BuLi [254] (see Figure 53 caption for the reaction conditions).

the second reaction stage can also be induced by increasing the *n*-BuLi concentration at a constant reaction time. The gel-permeation chromatogram (GPC) of a polymer sample after a reaction time of 7 min shows a broad polymodal molar mass distribution that indicates various interparticle reactions (Fig. 55). Primary particles, which are formed during the first reaction stage, appear after an elution volume V_e = 28–33 ml and the aggregate up to 28 ml. The maximum at V_e = 23 ml, corresponds to a $\overline{M}_w$ of 10×10^6 g/mol for EDMA microgels but to only 0.8×10^6 g/mol for linear polystyrene. Thus, the structure of EDMA microgels is about 12 times more compact then that of a linear polymers with the same hydrodynamic volume [254].

From the experimental data, Straehle concluded that polymerization and crosslinking proceed at first within individual macromolecules which are separated by solvent molecules. Reactive EDMA microgels are formed by these reactions. From the content of pendant methacrylate groups of the microgels, it can be calculated that about half of the structural units of the microgels are involved in cycles. As the reactions proceeds, the microgel particles come into contact with each other and the free volume of the reaction mixture decreases, thus allowing interparticle reactions. It can be calculated that the free volume disappears after a reaction time of 4 min at a conversion of 32%, which corresponds to the experimentally determined transition point from the first to the second reaction stage (Fig. 53).

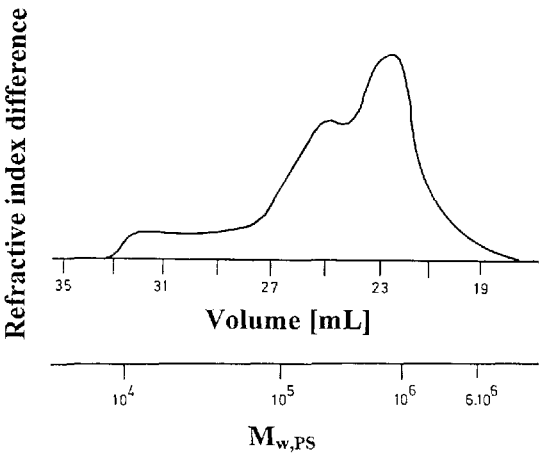


Fig. 55. Gel-permeation chromatogram(GPC) of a microgel sample of $\overline{M}_w = 10 \times 10^6$ g/mol obtained in the anionic polymerization of EDMA in toluene. Microgel concentration = 1 g/L; solvent = butyl acetate; elution temperature = 70 °C; is the weight-average molar mass of linear polystyrene used for comparison. [Reproduced from Ref. 256 with permission, Hüthig & Wepf Publ., Zug, Switzerland].

Pille et al. used living PBS chains to initiate the anionic polymerization of EGDM and 1,4-butanediol dimethacrylate. They obtained highly crosslinked microgels together with slightly branched oligomers of PBS of a low molar mass [260].

A comparison of the experimental data obtained in the anionic polymerization of EDMA, 1,4-DVB and 1,3-DVB shows the following characteristic features:

1) Almost linear polymers with pendant vinyl groups are formed as intermediates in the anionic polymerization of 1,4-DVB due to the different reactivities of monomers and pendant vinyl groups. 1,4-DVB microgels are formed towards the end of monomer conversion. In the anionic polymerization of EGDM or 1,3-DVB, reactive microgels are formed already at the beginning of the polymerization.
2) The content of pendant vinyl groups is 80–90% in case of 1,4-DVB microgels but only 50–70% and 50–60% in cases of 1,3-DVB and EDMA microgels respectively. The extent of cyclization increases in the order 1,4-DVB < 1,3-DVB < EDMA.

7.4 Microgels from Other Divinyl Monomers

Only a few publications have appeared in which for the synthesis of reactive microgels other monomers were used than 1,4-DVB or EDMA. Hiller and Funke studied the anionic polymerization of 1,4-diisopropenylbenzene (1,4-DIPB) by *n*-BuLi in 1,2-dimethoxyethane and by sodium naphthalene in THF [231].

Although the initial monomer concentration was very high (25–200 g/l), soluble polymers were obtained even after complete conversion of DIPB. The measurements of the content of pendant isopropenyl groups indicated that poly(4-isopropenyl-α-methyl) styrene was formed.

Okamoto and Mita studied the anionic polymerization of 1,4-DIPB in THF [261]. They found the reactivity of the pendant vinyl groups by about three to four orders of magnitude lower than that of the vinyl groups of the monomers. Popov et al. compared the reactivities of 1,4-DVB and 1,4-DIPB in the reaction with polystyryl dianions in THF/benzene mixtures [262]. While addition of 1,4-DVB to the dianion solution caused an immediate macrogelation, no gel formation was observed on the addition of 1,4-DIPB. Anionic polymerization of 1,3-DIPB was also studied by several research groups [263–265]. They reported formation of low molar mass species.

8 Other Techniques for Microgel Synthesis

The instability of the growing end in the anionic polymerization of methacrylates requires very low polymerization temperatures which limits the practical applicability of this method. As an alternative, group transfer polymerization (GTP) was developed by Webster and co-workers [266]. This method is called GTP because it involves the repeated transfer of a trialkylsilyl group from the growing end to the arriving radical [266–269]. Lang et al. initiated the polymerization of EGDM by using poly(MMA) chains with active end groups, synthesized via GTP, and prepared EDMA microgels enveloped by poly(MMA) chains [270]. Schoettner studied GTP of methacrylates carrying various photostabilizers, e.g. 2,2,6,6-tetramethyl piperidine derivatives [271]. The active copolymer chains formed in this way were then used to initiate the polymerization of EDMA. The microgels thus obtained exhibited efficient photostabilizer properties.

Another method of microgel synthesis is crosslinking of an AB diblock copolymer with incompatible chain sections in a so-called selective solvent that is a good solvent for one part of the block and a non-solvent for the other. Since the diblock copolymers form polymer micelles in selective solvents, crosslinking of the core yields microgels with crosslinked core and a soluble shell. Ishizu and co-workers prepared polyisoprene and poly(4-vinyl pyridine) microgels by crosslinking diblock copolymers poly(styrene-β-isoprene) and poly(styrene-β-4-vinyl pyridine) respectively [44, 272–274]. Another route of microgel synthesis was reported by Antonietti et al. [275]. They terminated polystyrene chains bearing two reactive end groups with a tetrafunctional crosslinking agent in dilute THF solutions. The polymers obtained in this way had a more compact structure than linear polystyrene chains.

Microgels may also be produced by dispersion polymerization of multifunctional monomers [276, 277]. Kim et al. synthesized microgels by copolymerization of acrylamide with acryloyl terminated polyethylene glycol macromonomers in ethanol or in selective solvents [276]. The macromonomer acted

both as crosslinking agent and steric stabilizer for phase separated particles. Kiminta et al. synthesized microgels by emulsifier-free dispersion polymerization of *N*-isopropyl acrylamide and *N,N'*-methylene bisacrylamide in water [53]. Capek and Funke studied the dispersion polymerization of this bisacrylamide and its copolymerization with unsaturated polyesters [278–281]. It was shown that the formation of microgel particles proceeds via aggregation in the separated particle phase. The presence of internal or external emulsifiers increased the stability of particles.

9
Surface Modification of Microgels

Reactive microgels are suitable substrates for topochemical reactions, such as grafting, copolymerization with other monomers or other chemical modifications. The reactivity of microgels may be introduced by pendant vinyl or other reactive groups which have remained unreacted during the synthesis or by choosing comonomers with chemically different reactive groups from which one kind does not participate in the formation of microgels, e. g. acrylic acid or carboxyl-terminated unsaturated polyesters.

For special purposes the reactive groups may also be modified after the synthesis of microgels. In this case the reactive groups should be readily accessible to the reagent and the conversion should be as high as possible, to avoid nonmodified groups and by-products that cannot be removed afterwards through being bound to the microgel. Sometimes several reaction steps are necessary for surface modification., e. g. aldehyde groups after their introduction to the microgel surface for binding proteins must be chemically blocked for protection and freed again before the coupling reaction.

A very important requirement for the chemical modification of microgels is the accessibility of the reactive group to the reagent [282] and the limitation of the modifying reaction to the surface. Therefore, besides the high specific surface area, microgels should be densely crosslinked in order to restrict the modification to their surface. Such microgels may be prepared from 1,4-DVB. The pendant vinyl groups located at the surface of these microgels react as rapidly as corresponding reagents of low molar masses, whereas the reaction of vinyl groups in the interior is controlled by diffusion and therefore much slower (Fig. 47). From these reaction rates may be calculated that about 60% of the pendant vinyl groups have been used for crosslinking, 5% are in the interior are not accessible and 35%may be used for modification.

With slightly crosslinked microgels it becomes increasingly difficult to distinguish between vinyl groups located at the surface and those in the interior because the reaction at the surface overlaps that in the interior. In addition to the influence of crosslink density and swellability of microgels, the dimension of the reagent is also a determining factor for the location of modifying reactions. The modifying reaction can only then be unequivocally assigned to the microgel surface if the reagent is larger than the meshes of the microgel network.

9.1
Reactions for Modifying and Characterizing Surfaces of Microgels

Topochemical reactions may serve both the modification and the characterization of the microgel surface. Moreover, the determination of the reactive groups of microgels is a prerequisite for judging the success of the modifying reaction. These modifying reactions may be needed for various purposes, e.g. increasing the resistance against chemical aging or introduction of functional groups for technical applications.

9.1.1
Characterization of Divinylbenzene Microgels

Because of their insolubility, the restricted access of chemical reagents and the influence of the neighborhood on the mobility of chain segments and functional groups of crosslinked polymers, the determination of residual reactive or functional groups in crosslinked polymers is much more difficult than in linear or branched polymers. This is especially true for densely crosslinked polymers prepared from tetrafunctional monomers, such as DVB.

Non-reacted vinyl groups of these crosslinked polymers may be expressed by the residual unsaturation (RU). The RU is a measure for both the reactivity of the monomer and the structure of the crosslinked polymer. The RU may be determined by spectroscopic or chemical methods. For the spectroscopic determination a model compound of low molar mass is required as a reference for the standardization [217, 231, 254]. For the chemical determination a reagent of low molar mass is added to the pendant vinyl groups. Then the RU is obtained either by elemental analysis or by back-titration of the non-reacted reagent [231, 283–285].

The RU may be measured by following methods:
- quantitative ^{1}H-NMR-spectroscopy [231];
- quantitative infrared spectroscopy [54, 217];
- catalytic hydration and volumetric measurement of the consumption of hydrogen [231];
- reacting with mercury(II) acetate [283];
- ionic addition of hydrogen bromide and analytical determination of the bromine content [284];
- radical addition of butyl mercaptan and elemental analysis of the sulfur content [285].

Comparing the results of different methods, it turned out that RU strongly depended on the respective method [284, 286].

Values for RU differed by up to 100% with 1,4-DVB-microgels [286]. The reliability of methods for determining the RU of 1,4-DVB-microgels was checked [287] with poly(4-vinylstyrene) which was prepared by anionic polymerization of 1,4-DVB (Table 3). From these results, it can be concluded that only quantitative IR-spectroscopy is a reliable method for determining the RU of 1,4-DVB-

Table 3. RU of Poly(4-vinylstyrene) determined with different methods

Poly(4-vinylstyrene) No.[1)]	RU [%] determined by		
	IR-Spectroscopy	ICl-Addition	HBr-Addition
1	100.7	92.1	85.2
2	100.3	92.8	87.0
3	98.8	92.0	88.0
4	99.8	92.5	81.6
5	102.3	93.0	86.3

1) Samples 1-5 have been prepared with different inititator concentrations (0.26 - 0.78 mol % based on the monomer)

microgels. The RUs of 1,4-DVB-microgels obtained with IR-spectroscopy [287] were significantly higher than those of the other methods which are obviously too low (Table 4).

9.2 Aging of Divinylbenzene Microgels

The pendant vinyl groups of DVB-microgels, like the monomers, are still reactive and susceptible to unintentional reactions leading to irreversible agglomeration or aggregation. Such aggregates may already be formed during isolation and purification of microgels. During exposure of reactive DVB-microgels in solid state to air, insolubility often develops after 1–2 days. The reason for this insolubility is radical reactions between pendant vinyl groups of neighbored microgel particles.

Table 4. The RU of 1,4-DVB-Microgels determined with different methods

Poly(4-vinylstyrene) No.	RU [%] determined by		
	IR-Spectroscopy	ICl-Addition	HBr-Addition
1	72	60	45.1
2	62	62.5	48.3
3	49	53	45.6
4	70	58	55.0
5	62	56	52.8
6	60	58	48.6
7	62	57	52.2
8	62	57	48.0
9	59	56.5	53.3
10	60.3	58.2	50.2

These interparticle reactions can be avoided by a sterical blockade of the reactive groups with the help of suitable comonomers [135] or by formation of a core-shell structure of microgels, by which the reactive groups are covered with a shell [244, 248]. It is also possible to add silanes to the vinyl groups [221]. By adding *n*-butyl mercaptan and small amount of initiator the pendant vinyl groups of previously isolated microgels may be completely saturated without changing the molar mass and viscosity of the microgels [285]. Thus, modified microgels are chemically stable and may be stored and handled without a change of their molar mass.

9.3 Introduction of Other Functional Groups in Microgels

The pendant vinyl groups at the surface of microgels can be modified in various ways according to the purpose of their application.

9.3.1 *Surface Modification by Hydroxy Groups*

By hydroboration [288, 289], pendant vinyl groups of microgels are almost quantitatively converted to hydroxyethyl groups [290]. Because the reagent has a small size it may also react with vinyl groups in the interior. Hydroboration of vinyl groups is faster than the reaction with mercury(II) acetate. Whereas the latter reaction is still not complete after 120 h, hydroboration is already quantitative after 24 h. After hydroboration, the surface properties of the microgels had changed and the microgels were insoluble in solvents in which they could be dissolved before. It was assumed that larger aggregates were formed, although not necessarily by covalent bonds because a redispersion to a colloidal solution was possible after an ultrasonic treatment [291].

9.3.2 *Surface Modification by Epoxide Groups*

For introducing epoxide groups, 1,4-DVB microgels were reacted with *m*-chloroperbenzoic acid. Unlike the conversion of vinyl groups to hydroxyl groups, only about 70 % of the vinyl groups could be converted to epoxy groups. The modified microgels were isolated from a non-aqueous solution to avoid agglomeration [291].

9.3.3 *Surface Modification by Ozone*

Vinyl groups of 1,4-DVB microgels have been converted to carboxylic acid groups by ozone [291]. After modification the microgels could be dissolved in methanol. About 83 % of the vinyl groups could be converted. A simpler way to prepare microgels with carboxyl acid groups at their surface is the copolymerization of DVB with methacrylic acid in an aqueous emulsion [292].

9.3.4
Surface Modification by Dye Molecules

For binding dye molecules to the surface of 1,4-DVB microgels, at first hydrogen bromide was added to the pendant vinyl groups and then a basic dye was reacted with the bromide group by a nucleophilic substitution [284, 293]. Table 5 shows the relationship between bromine content of microgels, conversion and amount of dye bound to the surface. The smaller the content of bromide groups, the larger was the fraction substituted by dye molecules. The decrease of the conversion has been explained by the hindrance of vinyl groups in the interior to react. Experiments with different nucleophilic dyes showed that the substitution depended on the basicity and on the dimensions of the molecules (Table 6). Whereas the brominated microgels could still be dissolved in benzene, dioxane or dichloromethane, for dye-modified microgels more polar solvents such as nitrobenzene were needed.

9.3.5
Modification by Polymer Analogous Esterification

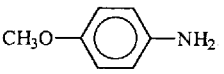
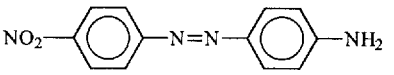
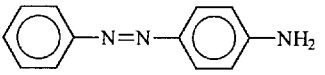
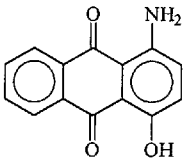
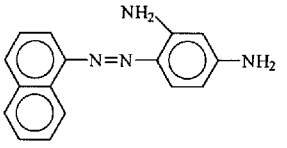
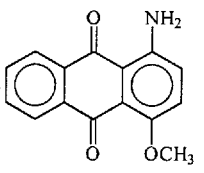
In self-emulsifying copolymerization of unsaturated polyesters and comonomers the terminal unsaturated groups of EUP are deactivated by the adjoining dissociated carboxyl acid group. By esterification of these acid groups the terminal unsaturated polyester units become active again. Moreover, an agglomeration of the microgels by hydrogen bonding between the particles may thus be prevented.

Ester formation by dimethylsulfate or diazomethane is not satisfactory because the microgels become insoluble when the reaction proceeds to higher conversions. With diazomethane part of the unsaturated groups is involved in a side reaction of a 1,3-dipolar cycloaddition [132]. A more efficient method for ester formation of microgels is the reaction with *O*-alkyl-*N,N'*-bisisopropyl isoureas of the alcohols. The alkyl ureas are easily separated from solutions in methanol [294–296]. The esterified microgels were isolated by precipitation and freeze-drying. Depending on the alcohol used for ester formation, the yields of

Table 5. Reaction of 4'-Nitro-1-aminoazobenzene with 1,4-DVB-Microgels of different bromide content.

Bromide content per unit [mol-%]	Conversion based on the bromide content [%]	Dye bound to the surface [mg /g polymer]
9.5	57	99
13.5	46	116
22.1	43	177
27.9	37	193

Table 6. Reaction of HBr-modified 1,4-DVB microgels with different dye molecules. (Br-content: 2.1 mmol/g, ∅ : 9 nm, specific surface: 630 m^2/g, reaction conditions: 40 h, 60 °C, solvent: ethanol)

Nucleophile	Conversion based on the bromide-content [%]	Bound nucleophile [mmol/g]
	45.0	0.94
	37.5	0.79
	36.0	0.76
	33.0	0.69
	26.0	0.55
	18.5	0.39

esterified microgels were between 60 and 80% [297]. Esterified microgels with short-chain alcohols are soluble in dioxane. Microgel esters with longer-chain alcohols may be dissolved in a mixture of dioxane and water. In all cases ester formation was quantitative, although molar masses and particle diameters indicated that some soluble agglomerates had been formed.

9.4 Synthesis and Modification of Microgels for Biochemical Purposes

As carriers for proteins and enzymes biocompatible reactive microgels must be synthesized which are soluble in the serum at 37 °C. Moreover they should be hydrophilic enough that no ionic monomers are needed but they should not be soluble in water. An inert comonomer should serve as a spacer as well as a reactive solvent that may dissolve solid comonomers. The coupling reaction should be possible under mild reaction conditions.

For using microgels as carriers of biological materials specific chemical groups (e.g. aldehyde groups) must be available at their surface which guarantee mild reaction conditions in aqueous media for coupling biomaterials. For this purpose, microgels must be soluble in water and their surface should be hydrophilic. Moreover, for diagnostic purposes DVB microgels are too small. The microgels have been prepared by copolymerization of functional comonomers in solution and in emulsion.

9.4.1 *Functional Comonomers*

Aldehyde groups are useful for binding proteins to polymers, e.g. via ϵ-lysine amino groups. However, the formation of semi-acetals and the oxidation of aldehyde groups during polymerization impose some problems. To avoid the formation of semi-acetals either copolymerization with an inert monomer as "spacer" or the use of a monomer with an aldehyde group at its "spacer arm" is indicated. Aldehyde groups can be protected by acetal formation [298, 299]. For aqueous ECP, monomers are needed which are insoluble in water such as di-*n*-pentylacetals [291]. These acetals are stable during ECP. A disadvantage of the acetal groups is the fact that they cannot be partially transformed into aldehyde groups necessary for binding proteins. Therefore, it is not possible to study how the bound molecules influence the residual bioactivity of bound enzymes. A possibility to vary the number of aldehyde groups in a polymer by choice is their copolymerization with acrylic- or methacrylic acid-2,3-epoxy propylesters [300–307]. However, most enzymes are denaturized because most functional groups of the proteins react with epoxide groups. Because glycidyl methacrylate causes some additional problems [291], N-substituted acryl- and methacrylamides have been synthesized with a 1,3-dioxolan group which is a protected diol group. These comonomers are not soluble in water, and after the ECP the dioxolane ring is easily opened to form the diol [308].

Microgels prepared in that way are hydrophilic, stable and do not tend to agglomerate. By oxidation of the diol group with sodium periodate a free alde-

hyde group is formed which is firmly bound to the microgel surface. As crosslinking comonomers, bisacryl- and bismethacrylamides are suitable [291].

9.4.2
Copolymerization in a Homogeneous-Aqueous Solution

For this reaction, soluble monomers are needed, e.g. a mixture of *N N'*-methylene bisacrylamide as crosslinker, methacrylamide as an inert comonomer, methacrylic acid as ionic comonomer for stabilization [309] and methacryl amido-*N*-acetaldehyde-dimethylacetal as functional comonomer. The coupling with proteins is only possible if the free aldehyde groups are accessible, i.e. if they are not located in the interior of the microgel. This condition can only be fulfilled by a careful choice of the comonomer composition in the reaction mixture [291].

Compared with rigid microgels, the intrinsic viscosity of microgels prepared from the comonomer mixture mentioned before is higher, but the slope of the curve in Fig. 56 is still low because the composition of these microgels was close to the limit of stability.

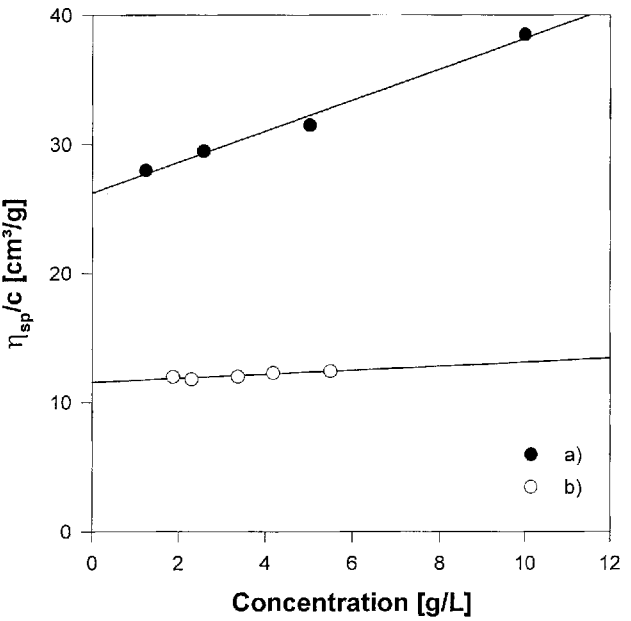


Fig. 56. Dependence of $\overline{M}_w$ of the microgels on the polymer yield in the anionic polymerization of EDMA in toluene by n-BuLi [254] (see Figure 53 caption for the reaction conditions). Reduced viscosity vs concentration of microgels a) Composition (mol %): N,N'-methylenebisacrylamide (55%), methacrylamide (33%), methacrylic acid (2%), methacrylamido acetaldehyd-dimethylacetal (10%), measured at 20 °C in water. b) Composition (mol %): 1,4-DVB (35%), propenic acid amide-2-methyl-N-(4-methyl-2-butyl-1,3-dioxolane prepared by emulsion copolymerization and measured in dimethylformamide.

9.4.3
Copolymerization in an Aqueous Emulsion

Microgels as carriers of biomaterials may also be prepared by copolymerization in an aqueous emulsion. For this purpose, besides the crosslinker a functional comonomer is used which, in addition to a polymerizable vinyl group, also contains a precursor for the aldehyde function. Microgels from ethylene dimethacrylate and methacrylic acid-2,3-epoxypropylester in a molar ratio of 2:3 had a mean diameter of around 50 nm and a specific surface area of 107 m^2/g [291]. After opening of the epoxide ring these microgels are rather stable. After purification, colloidal solutions in water or in mixtures of water and ethanol, dioxane or acetone are obtained. These microgels are sufficiently hydrophilic to allow coupling with various proteins under mild reaction conditions.

Microgels prepared by aqueous ECP of DVB and propene-acid amide-2-methyl-*N*-(4-methyl-2-butyl-1,3-dioxolane [308] had a molar mass of $\overline{M}_w$ = 1.4×10^7 g/mol and a mean particle diameter $\overline{d}_z$ = 66 nm. These microgels have a compact structure with a coil density in water of 0.16 g/cm^3 and an intrinsic viscosity [η] = 11.8 cm^3/g with a very low slope of the η_{sp}/c-curve (Fig. 56) [291]. After splitting off the protective acetal groups, very stable aqueous solutions of microgels are obtained. After proteins are coupled to such microgels, the -C=N-group has to be reduced to a -CH-NH-group.

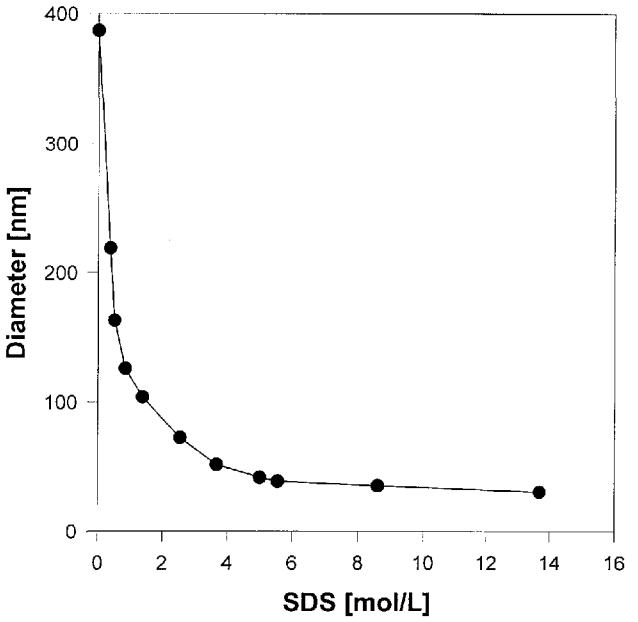


Fig. 57. Diameters of microgels prepared with different emulsifier concentrations (SDS). Composition (mol %): N,N'-tetramethylenebismethacrylamide (10%), N-n-hexylmethacrylamide, propenic acid amide-N-(4-methyl-2-butyl-1,3 dioxolane (50%)

A microgel of a $\bar{d}_z$ = 76 nm which is suitable for coupling with proteins, can be prepared by emulsion terpolymerization of *N,N'*-tetramethylene bisacrylamide, *n*-hexylmethacrylamide and propene acid amide-*N*-(4-methyl-2-butyl-1,3-dioxolane) [291]. The diameter of these microgels may be varied by the concentration of the emulsifier (Fig. 57) and is rather uniform. As the CMC of this system is about 2.5×10^{-3} mol SDS/l, it may be assumed that below this value the copolymerization essentially takes place in the monomer droplets, whereas at higher concentrations of SDS preferentially the monomers in micelles are polymerized.

10 Applications of Microgels

Discussing applications of microgels for industrial purposes, it is interesting that microgels are formed unintentionally in the synthesis of elastomers and alkyd resins. Due to the presence of potentially crosslinking isoprene and butadiene units in elastomers some intramolecular crosslinking takes place, probably also involving radical transfer reactions [6]. The detection, isolation and characterization of these microgels in elastomers has been reported [310–312], as well as their influence on the mechanical properties of the elastomers [313,314] and the conversion of microgels to macrogels [315].

Microgels have also been detected as a component of alkyd resins, an early but still important binder of organic coatings [316–321] and are accountable for their ability to fill pores, fissures and other irregularities of the substrate such as wood. This property may be explained by the size of the microgels which prevents the paint becoming soaked up by the substrate.

These examples show that microgels already played a role in the properties of important industrial polymers before they were intentionally added as a component. The more significant applications of microgels may be summarized as:

1) components of binders for organic coatings;
2) carriers of dyes, pharmaceuticals and biochemical compounds;
3) fillers and materials for reinforcing plastics.

10.1 Organic Coatings

The most important industrial application of microgels are organic coatings where they serve as a component of the binder. The advantage of microgels in the formulation of paints is their low viscosity. This property which allows formulations with high contents of solids have microgels in common with latex particles obtained by normal emulsion polymerization of bifunctional monomers. However, due to the much smaller size and compact structure of microgels, they can be dissolved in water or in organic solvents to form colloidal solutions whereas latex particles are only dispersible as emulsions or latices. Binder compositions containing microgels are often rather complex in order to comply with the requirement of application and performance.

Probably the first suggestion to use microgels, especially reactive ones, for organic coatings goes back to 1977 [322] when it was indicated that they might be suitable for preparing paints with high contents of solids and of low viscosities [323].

Crosslinked acrylic microgels in aqueous and non-aqueous media were patented as paint constituents in 1979 to improve the orientation of aluminum flake pigments, restrict the flow of the liquid coating on the substrate and restrict sagging [324]. As the patent speaks of emulsions, insolubility of the microgels and particle sizes up to 200 nm, it is questionable whether these polymers consisted of microgels only.

The industrial production and application of reactive and non-reactive microgels in organic coatings such as binders or components of binders, e.g. together with, e.g. acrylic and/or melamine/formaldehyde resins, especially for automotive coatings, was reported in a number of publications between 1980 and 1990 [325–333].

Special properties and advantages of using microgels as binder component for both aqueous and non-aqueous paints and coatings such as the decrease of sagging, the orientation of flake pigments, the increase of tensile strength, the low viscosity, the adjustment of the rheological behavior (Newtonian or pseudoplastic flow, depending on the microgel concentration), the increase of abrasion resistance, antiblocking properties, control of surface properties, the reduction of shrinkage, an increase of the permeability for water where needed, and an adjustment of the hiding power have been mentioned as the benefits of special paint formulations [334–338].

Acrylic microgels can be prepared as non-aqueous dispersions (NAD) and aqueous dispersions for the formulation of high solid paints for basecoats [339, 340]. The intramolecular crosslinking was achieved by the addition of triethylenediamine which reacts with linear acrylic terpolymers containing glycidyl methacrylate units or by incorporation of allyl methacrylate or hexamethoxymethylmelamine. Such microgels assist the rheological control during the application of thermosetting acrylic metallic finishes by improving the alignment of flake pigments which is needed to obtain the "flop effect" characteristic of metal effect coatings.

Other NAD microspheres are composed of styrene, MMA, hydroxyethyl acrylate, acrylic acid and acrylonitrile and are blended with acrylic copolymers and melamine/formaldehyde resins [341, 342]. Particles of this polymer are used as rheology modifiers to prevent sagging in automotive coatings and for controlling the orientation of metal flake pigments.

However, some doubt exists whether these dispersions really contain microgels only because their insolubility was emphasized and the range of particle size mentioned was up to 10 μm.

The question whether the intramolecularly crosslinked microparticles of non-aqueous polymer dispersions are really microgels is also justified, considering non-aqueous dispersions prepared from acrylic copolymers and melamine/formaldehyde crosslinker with particle sizes of about 300 nm. [45, 343]. In any case, these crosslinked polymeric microparticles are useful constituents of high-solids coatings, imparting a yield stress to those solutions which probably involves attractive forces between the microparticles.

Use of NAD microgels with a low glass transition temperature improved the mechanical performance, durability and resistance against blistering of coatings for household and industrial buildings [344, 345].

Crosslinked polymer particles with a rather complex structure, which have also been designated by the name microgels and recommended as components of metal effect paints, consist of carboxyl-terminated oligoesters of 12-hydroxy stearic acid which were reacted with glycidyl methacrylate, subsequently copolymerized with MMA and hydroxymethyl methacrylate and then crosslinked by hydroxy melamine [346].

Microgels with an acrylic core for waterborne base coats have been reported to resist the attack of subsequent clear coats, exhibit mechanical toughness and flexibility and have a good durability and chemical resistance [347].

Rheological properties of microgels used in automotive coatings have been reviewed and discussed in [348].

Following the knowledge of microgels as constituents of alkyd resins, microgels have been prepared from a maleinized alkyd resin which was copolymerized and crosslinked with 1,6-hexanediol diacrylate. [349]. Coatings from these microgels have increased, and are harder and more resistant to water. When blended with water-soluble resins air-drying coating materials are obtained which can be applied by airless spraying and give coatings with increased tensile strength.

Microgels can not only be synthesized by polymerization but also by polycondensation or polyaddition [350]. In an early work on crosslinking of single linear macromolecules, it could be shown that if a crosslinking agent, such as terephthal dialdehyde, was added to a very dilute solution of a linear polymer such as polyvinyl alcohol, almost exclusively a intramolecular crosslinking of the individual macromolecules took place [351].

Colloidal particles have been detected in thermosetting resins [352] and the production of particulate phenolic resins, albeit of larger sizes than microgels, has also been reported [353].

Microgels have been prepared from epoxy resins which were intramolecularly crosslinked by a polyalkylene polyamine/polycarboxylic acid for flexible, corrosion resistant coatings [354].

Microgels have been also synthesized using isocyanates or polyurethanes [355, 356] and by polycondensation of silanes [357–360]

10.2 Microgels as Carriers for Dyes

Due to their large surface area and the reactive groups located there, microgels may be used as carriers [362] and substrates for various purposes. The idea to bind dye molecules covalently to surfaces was realized first with reactive dye molecules [322]. Functionalized dye molecules were copolymerized by radicals with other monomers to obtain colored plastics, from which no dye molecules could migrate. Likewise it is possible to bind organic dye molecules covalently to the surface of microgels, thus obtaining colored organic pigments [284, 361–363].

Larger crosslinked polymer particles were prepared earlier for application as pigments [364].

For the synthesis of these pigments the dye molecules must possess a high light fastness. The colors are not very bright, and because of the thin dye layer these pigments are more susceptible to an oxidative photodegradation than normal pigments.

10.3 Microgels as Substrates for Biomedical and Diagnostic Purposes

Microgels may be used as substrates and carriers for enzymes and antibodies [291, 308, 365–371]. These agents may be covalently bound to the surface of suitable microgels and thus may easily be separated from the reaction mixture. As the amount of the reagent can be used in excess, the reaction equilibrium is shifted to the products and higher yields are obtained. For some diagnostic and therapeutic applications such as drug targeting, it is necessary that carriers have small, submicroscopic dimensions in the range of nanometers [372–374]. Therefore, microgel with sizes below 100 nm are specially suitable for these purposes. For such small particles the reticular endothelial system is penetratable.

Proteins may be bound to microgel surfaces by various reactions directly or via a spacer [375]. A suitable group, which can be introduced to the microgel surface, is the aldehyde group which reacts with the amino group of a protein. Then the resulting azomethine is reduced (see Sect 9.4). For modifying the microgel surface with aldehyde groups, they must be intermediately protected.

To avoid losses of the protein activity and to increase the stability of the microgel the protein may be coupled with a spacer, e.g. enzymatically cleavable oligopeptides. Like in the case of direct coupling, the oligopeptide is first bound to the microgel surface by the reaction between an aldehyde group of the microgel and an amino group of the oligopeptide. After the reduction, the protein is bound to the spacer by the reaction of its acid group with the amine group of the protein. For this reaction the carboxyl acid group is activated by carbodiimide [308]. The use of a spacer also prevents a direct contact of the protein with the microgel surface and thus denaturation. Moreover the active site of the protein is more easily accessible.

The amount of enzyme which can be bound to microgels depends on the structure of the enzyme. Enzymes with a higher isoelectric point are better bound to negatively charged microgels. It is also possible to bind sensitive enzymes such as lactate dehydrogenase or proteinase K to microgels with high yields and high residual activity [365, 366, 375].

An immuno assay for α-1-fetoprotein has been developed with microgels by binding antibodies to their surface [291, 375]. With corresponding antigenes in solution these microgels aggregate to form much larger particles which can be detected by photon correlation spectroscopy.

An essential prerequirement for the aggregation is the presence of different epitopes on the antigenes so that their functionality is greater than one. Reversible bridging flocculation of poly(lysine) with acrylic microgels has also been reported [376].

10.4 Microgels as Fillers

Reactive microgels may be incorporated into plastics by covalent bonds. It could be demonstrated that substantial amounts of polymer chains from bifunctional monomers can be attached at microgel surfaces and thus become insoluble [313, 377–380].

An interesting way to prepare shock-resistant coatings [381] follows the synthesis of the ABS-terpolymers, e.g. shock-resistant polystyrene, where a soft, elastomeric phase is incorporated in a hard polymer matrix via covalent bonds. Because organic coatings solidify in situ, elastomeric microgels have been synthesized and mixed to a binder which forms the hard matrix phase before the application of this mixture as a coating material.

Epoxy resins have been toughened by in situ copolymerization of microgels consisting of unsaturated polyesters and bifunctional comonomers [382, 383].

11 Concluding Remarks

For a long time crosslinking reaction steps in the polymerization of unsaturated monomers have been considered to lead inevitably to insoluble polymer materials, even with small amounts of the crosslinking component. Moreover, small crosslinked polymer particles were a nuisance in the production and characterization of polymers as unpredictable products of side reactions.

Experimental and analytical studies over the past 25–30 years revealed that microgels are intramolecularly crosslinked macromolecules, which represent a new class of polymers besides linear and branched macromolecules and crosslinked polymers of macroscopic dimensions. In some ways microgels may be considered as a transition from molecules to larger polymer particles or macroscopic polymer materials.

Microgels are distinguished from linear and branched macromolecules by their fixed shape which limits the number of conformations of their network chains like in crosslinked polymers of macroscopic dimensions. The feature of microgels common with linear and branched macromolecules is their ability to form colloidal solutions. This property opens up a number of methods to analyze microgels such as viscometry and determination of molar mass which are not applicable to the characterization of other crosslinked polymers.

Similar to macroscopic polymer networks, microgels have a more or less fixed surface. Due to the large value of their surface/mass ratio, microgels may be used as models for studying topochemical reactions at polymer surfaces.

The reactivity of microgels depends on the kind and composition of their monomer components and can be varied over a wide range. The reactive groups of microgels at their surface are useful for modifying reactions but also make them susceptible to interparticle crosslinking which leads to the formation of insoluble and irreversible agglomerates or aggregates. By careful choice of polymerization conditions, the formation of larger, insoluble polymer particles can be avoided.

The mechanism of crosslinking emulsion polymerization and copolymerization differs significantly from linear polymerization. Due to the gel effect and, in the case of oil-soluble initiators, monomer droplets polymerize preferentially thus reducing the yield of microgels. In microemulsion polymerization, no monomer droplets exist. Therefore this method is very suitable to form microgels with high yields and a narrow size distribution, especially if oil-soluble initiators are used.

Microgels which have been prepared in emulsions or microemulsion have a more compact structure than those obtained by polymerization in solution. For microemulsion copolymerization, preferentially self-emulsifying comonomers, such as unsaturated polyesters, are used as polymerizable surfactants, because no emulsifier must be removed after the reaction. By choosing suitable monomer combinations the composition, size and structure of microgels can be widely varied, thus adjusting these macromolecules to special applications.

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